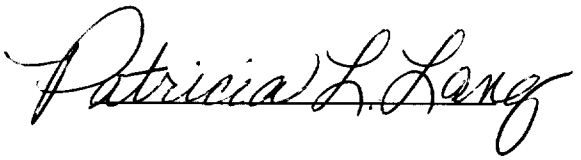
Non-Destructive Measurement of Gelatin Content of European Papers: 1400-1800

An Honors Thesis (HONRS 499)

by

Brenda S. Fuller

Patricia Lang, Advisor



Ball State University

Muncie, Indiana

Date of Graduation
May 4, 1996

Purpose of Thesis

The broad purpose of our research is to determine the effect of gelatin concentration on paper permanence, in collaboration with paper scientist Tim Barrett of the University of Iowa. Our specific research goal is to use attenuated total reflectance (ATR) infrared spectroscopy and various factor analysis methods to characterize historical papers in a non-destructive manner. We have tested and developed the infrared sampling techniques and have compared several factor analysis methods to determine a method which can best be used to analyze properties of the paper samples. The method that proved most useful allows gelatin properties to be estimated by their infrared spectras, provides accurate predictions of sulfur and potassium content, and estimates the paper's date.

INTRODUCTION

HISTORY OF PAPER-MAKING IN EUROPE

In Europe between fourteen hundred and eighteen hundred, gelatin was commonly used in the paper-making process for several reasons. Gelatin was used as a sizing agent, and it helped prevent bleeding of the ink. Gelatin also increased the strength of the paper and made the paper more resistant to abrasion. Some studies suggest gelatin acts as a buffering system against environmental pollutants that catalyze the acidic degradation of paper. It is still unclear what effect, if any, gelatin has on paper permanence.

FIRST STUDIES OF GELATIN CONTENT OF HISTORICAL PAPERS

Tim Barrett of the University of Iowa originated the study of the effect of gelatin on paper permanence. He took forty historical papers and divided them into two groups according to their light values that were measured using a Minolta Chroma-meter. Surface pH, calcium content, iron content, sulfur content, and potassium content, as well as the gelatin content were all measured. The gelatin content, or percent hide glue, was determined by the concentration of 4-hydroxyproline. A significant correlation between these values and the light values was found, indicating that these properties aid in the preservation of paper. This also means that gelatin may aid in the preservation of paper. However, in order to validate this notion, a non-destructive method for determining gelatin for a larger number of samples would need to be employed. This is the primary purpose for our study.

EXPERIMENTAL METHODS

PRINCIPLES OF ATR

We have chosen an infrared sampling process known as attenuated total reflectance (ATR). ATR is a non-destructive method of internal reflectance spectroscopy, meaning that the paper sample is not destroyed when the spectrum is taken. The paper sample is placed directly

against two sides of a crystal with a high refractive index (See Figure 1). A KRS-5 element was used in this research. Complete internal reflection occurs at the interface of the sample and element, given that the angle of incidence is less than the critical angle. Some radiation inevitably penetrates the surface of the sample, and the sample absorbs particular frequencies. These absorptions provide the infrared spectra and characteristic peaks.

Gelatin has several characteristic infrared absorption bands (See Spectrum 1). The main two bands are the amide I band and the amide II band. The amide I band is caused by the C=O stretching of the amide bonds (found at approximately 1650 cm^{-1}), and the amide II band is a result of the C-N-H bending (found at approximately 1550 cm^{-1}).

PROCEDURE

First, acceptable spectra from both sides of each sample had to be taken using the ATR accessory coupled to a Perkin-Elmer 1760X FT-IR spectrometer. Care had to be taken so that enough pressure was applied to the sample to obtain a good spectrum without fracturing the crystal. The spectra from the two sides of the sample were averaged together to decrease the effect of the inhomogeneities of the paper.

The spectra of 25 of the 40 historical paper samples were then run through the statistical package (Quant +, Perkin-Elmer software), principle components regression (PCR) as a calibration set. (The remaining 15 samples were not used because visible evidence showed damage sustained during previously performed pH measurements.) The PCR algorithm attempts to express the variation in the spectral data in as few terms as possible. PCR takes the raw spectral data and breaks it down into a set of weighted factors. Then, the computer drops those factors that do not correlate to the given properties. This produces a set of factors that are then compared to the properties of the standard set. Factors may be either positive or negative. The first factor is approximately the weighted average of all the original spectra. The rest of the factors account for the residual variation in the data set (See Figure 2).

When the significant factors are recognized, their contributions to the calibration spectra are calculated. These contributions are called the factor loadings. A set of loadings corresponds to the contributions of each factor to its calibration spectrum. Multiple linear regression is then used to identify correlations between the factor loadings and the properties of the samples. Factors that show little correlation to a property are eliminated until the remaining factors show a significant correlation. By using graphs, known values of properties in the calibration set are compared with those predicted by the regression equation. The linear regression of the graph provides a correlation factor for the set.

To generate the best model possible, the expert assist option was used for our models. Expert assist automatically rejects abnormal or eschewed standards until the best possible model is generated.

RESULTS AND DISCUSSION

Graph 1 shows the correlation for the computer's predictions of gelatin concentration from the regression equation vs. the "known" gelatin concentration obtained by the wet chemical hydroxyproline method. From a calibration set of 25 historic paper samples, we have a multiple correlation of 0.8057, a significant R value considering the calibration set was constructed with a wide variety of paper samples of different natural origin. In addition, we find that five factors were extracted from the set of 25 spectra and three correlate with gelatin. In addition, since our collaborators had characterized each of these papers with regard to surface pH, calcium, sulfur, iron, potassium, fluorescence, and light, we entered these values for those properties into our statistical package and did a regression to see if any extracted factors correlated with them. This is shown in the lower portion of Table 1. We can see that sulfur, potassium, and date correlate with an R value of about 0.7 or higher. In addition, or perhaps more interesting, some extracted factors are common among different properties. For example

factor four and five correlate with gelatin, sulfur, and potassium. We speculate that this relationship might be related to the fact that alum (unalum potassium sulfate) was often used in conjunction with gelatin sizing.

To validate this method, the factor analysis package performed a full cross-validation test on the data set. Using information from the validation report, graphs of estimated validation values vs. specified values were constructed for each property (See graphs 2-10). Gelatin, sulfur, and potassium concentrations showed high correlations, indicating that this method showed promise for the predictions of these properties.

In order to evaluate the effect aging had on our prediction capabilities, we analyzed a set of three papers from an earlier study: a Whatman filter paper, the same paper after gelatin sizing, and the same paper gelatin sized after accelerated aging (exposure to auto exhaust followed by heat). We obtained spectra using the same procedure as outlined for the historical papers in our calibration set, then used the factor analysis model previously developed. The results can be seen in Table 2: IR Predicted Properties of Artificially Aged Filter Papers.

There is essentially no difference between the aged and unaged samples when we estimate the error in our ability to calculate gelatin concentration as being approximately equal to the concentration calculated for the 0% gelatin sample. This implies that the spectral characteristics detected in our method that are not connected with gelatin are not changed by the aging method. The properties of L^*, pH, date, and calcium all show little differences between aged and unaged samples as well. It should be noted that there is a significant difference in the L^* values of the paper with no gelatin and the papers with gelatin.

In an attempt to increase prediction capabilities, a new calibration set was created by adding known flax standards. The flax samples included flax with 0% , 0.5%, 1.0%, 2.0%, 4.0%, 6.0%, and 8.0% gelatin. After the new calibration set was run through the factor analysis program defined earlier, factors one and two were plotted against each other since they

are the two factors that show the most variance. Circles were drawn around each cluster of data points, and only one data point was chosen from each cluster. (See figure 3). This provided a new calibration set, with a more even distribution of values, making the data less weighted. Graph 11 shows an estimated vs. specified plot for gelatin concentration after the flax samples were combined with the 25 paper samples, and after the "clustering" procedure was performed on the new set. The R value for gelatin was found to be 0.9298. The R values for the other properties are as follows: pH, 0.4560; L^*, 0.6020; calcium, 0.7227; sulfur, 0.8665; potassium, 0.5391; iron, 0.9985; fluorescence, 0.9687; and date, 0.8165. (See Table 3 for a breakdown of each of the contributing factors.) The samples deleted as a result of the clustering method were then used as test samples to validate the computer's ability to accurately predict gelatin content and the other properties using our new calibration set. This information was converted into graphs showing estimated vs. specified values for each of the properties. In some of the graphs, outliers decreased R values, so they were removed. The R value of gelatin for this validation set was 0.27925 (See Graph 12). After the outlier (Sample 117T) was removed, an R value of 0.62835 was obtained (See Graph 12B). The other R values are as follows: pH, 0.17543 (See Graph 13); L^*, 0.20006 (See Graph 14) - After the outlier (Sample 82T) was removed, an R value of 0.50562 was obtained (See Graph 14B); calcium, 0.23225 (See Graph 15) - After the outlier (Sample 82T) was removed, an R value of 0.37593 was obtained (See Graph 15B); sulfur, 0.43382 (See Graph 16); potassium, 0.55501 (See Graph 17); iron, 0.30236 (See Graph 18); fluorescence, 0.55588 (See Graph 19); and date, 0.29721 (See Graph 20) - After the outlier (Sample 157W) was removed, an R value of 0.70115 was obtained (See Graph 20B).

The flax samples were then removed from the "clustered" calibration set to observe the effect of the flax samples in our new method. The R value of gelatin was significantly lower (R=0.3614. See Graph 21) for this data set than the set with the flax samples added

band (See Spectrum 1). The relative pathlength of the standards were then determined from the reference band.

CONCLUSIONS

The use of factor analysis allows an automated, objective method of extracting the spectral features which are responsible for the variance among the samples in the calibration set. These features may not be apparent visually to the analyst, are often overlapped, or are affected by baseline variations, noise and impurities. Once a method's parameters have been established, sample prediction is simplified greatly. Now, the analysis can be performed repeatedly to analyze other samples. The multiple linear regression allows more than one factor to be used in the calculation of a property value. This usually improves the results. Overall, factor analysis has the potential for use as a non-destructive and simultaneous method of determining several properties: pH, calcium, etc. Factor analysis also shows promise for providing insights on the relationship between various paper constituents.

It can be concluded that instruments that would analyze the pH of papers without causing as much damage as current methods would improve the results. Portable instruments for measuring ultrasonic wave propagation, infrared, and x-ray fluorescence would be very helpful in the non-destructive determination of these properties of historical papers as well. The horizontal ATR accessory shows promise for a more non-destructive method of analyzing the papers. The paper can be placed directly on the crystal, and clamped into place. This means that the paper does not need to be cut. Also, the clamp is calibrated, so the pressure on each sample can be equal. This allows for better reproducibility. We have not done intensive research using this accessory, but a few samples were examined, and the spectrum obtained appear to be acceptable as well. Therefore, this study deserves further research.

(R=0.9298). The R values for pH, calcium, and date were lower than the previous set as well (See Table 4 for a breakdown of each of the contributing factors). This method was then evaluated in the same manner as with the method that included flax. The information obtained was converted into graphs showing estimated vs. specified values for each of the properties. The R value for gelatin for this calibration set was 0.23975 (See Graph 22). The other R values are as follows: pH, 0.08476 (See Graph 23); L^*, 0.10065 (See Graph 24); calcium, 0.0013642 (See Graph 25); sulfur, 0.35944 (See Graph 26); potassium, 0.53877 (See Graph 27); iron, 0.35923 (See Graph 28); fluorescence, 0.45948 (See Graph 29); and date, 0.38299 (See Graph 30).

The testing of the three methods shows that there is not a significant difference in the first two methods. The validation tests indicate that both of these methods allow a reasonable estimated of gelatin, sulfur, and potassium concentrations. The clustering seems to improve on the date predictions, while decreasing the accuracy of the sulfur and potassium predictions. The last method showed a significant decrease in the prediction capabilities of most all the properties. Overall, our validation tests show that the first method is generally the most useful.

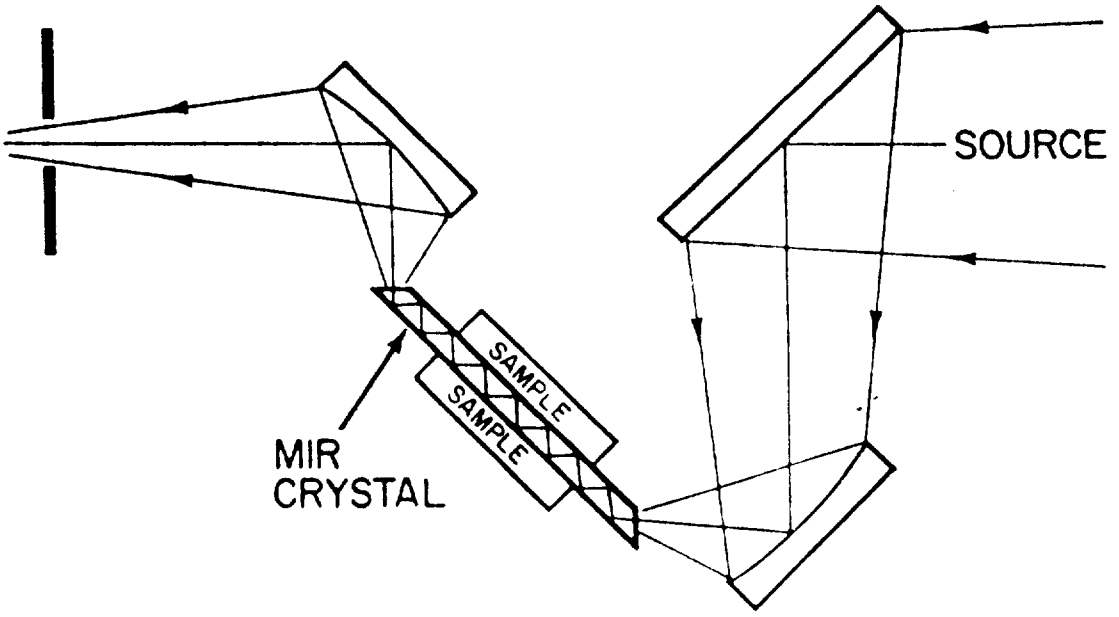
Throughout our research, inhomogeneity within a given sample has limited the reproducibility of our predictions. Since one side of a sample might have a higher gelatin content than the other, we compensated by averaging the spectrum from each side together.

The other factor that affects reproducibility is the penetration depth the radiation. This penetration depth differs between samples for two reasons. The surfaces of the samples are different, and the pressure applied to the sample against the crystal is also variable. Since the pathlengths of the standards are unknown, normalization parameters were adjusted to overcome thickness variations by using the cellulose absorptions from 900 to1250 cm^{-1} as a reference

ACKNOWLEDGEMENTS

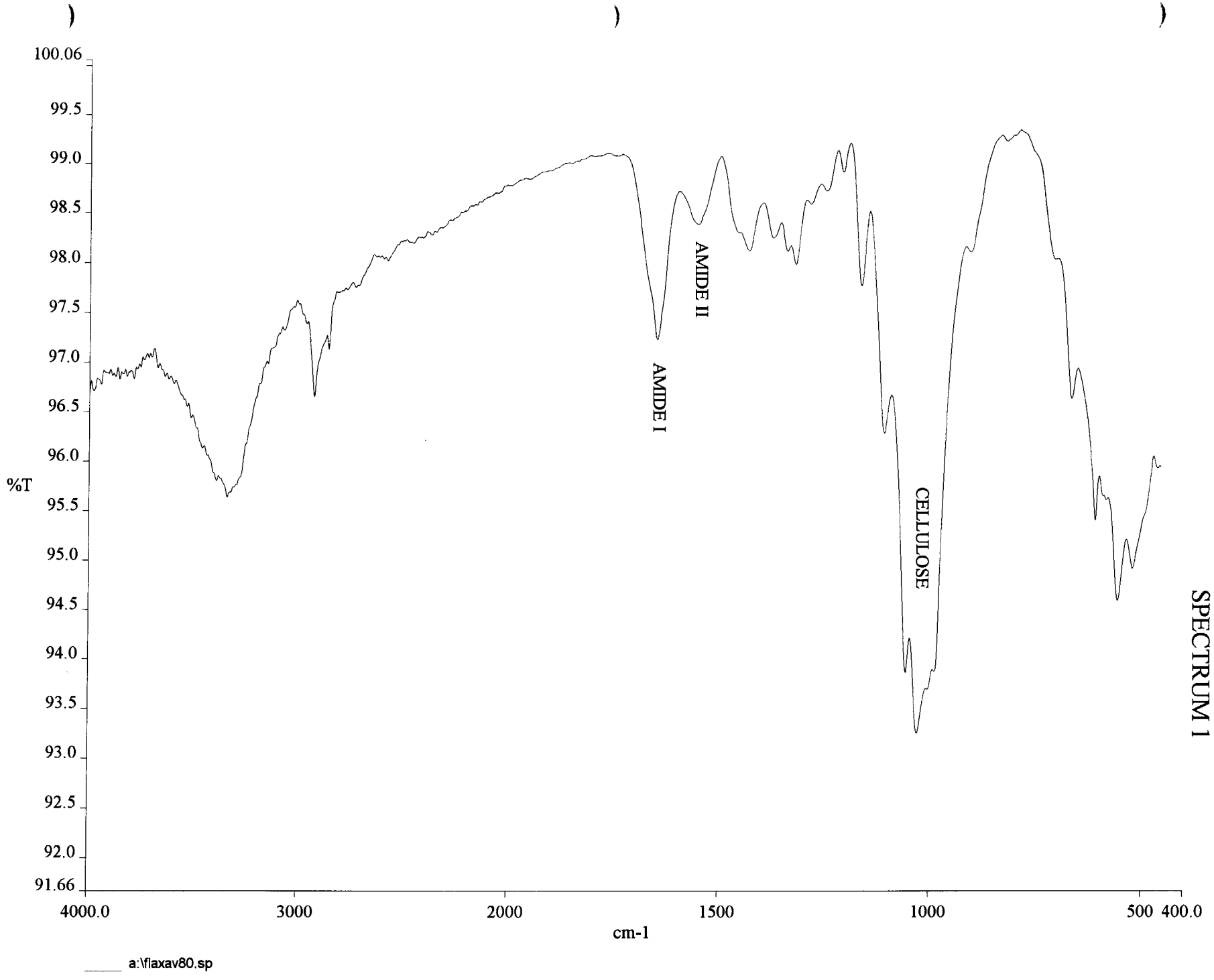
I would like to take this opportunity to thank everyone who has helped me in any way with my thesis. First, I would like to thank my advisor Dr. Lang for all of the guidance she has given me. Her excellent teaching abilities triggered my interest in research. I am grateful to Timothy Barrett for initiating the research on historical papers. I would also like to thank the Honors college undergraduate fellowship program for providing funding for our research. Finally, I would like to thank my parents and my family for supporting me in every way, and for always believing in me.

Attenuated Total Reflection (A.T.R.)

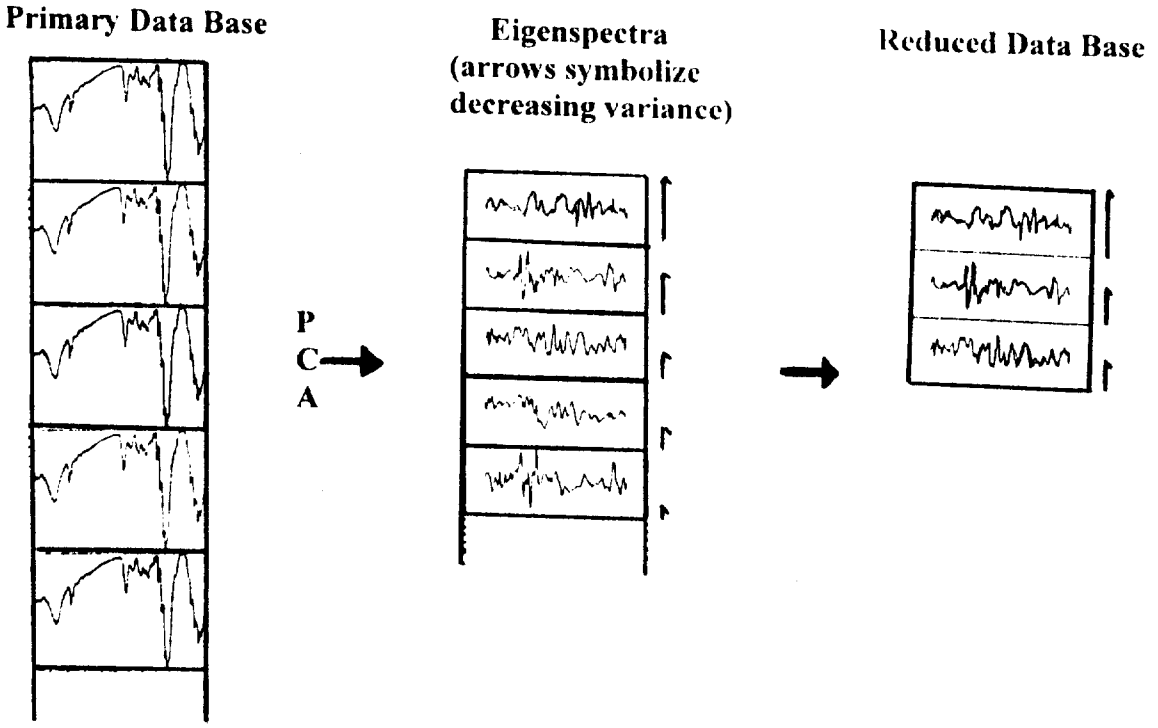


Optical diagram of a typical internal reflectance accessory.

Figure 1

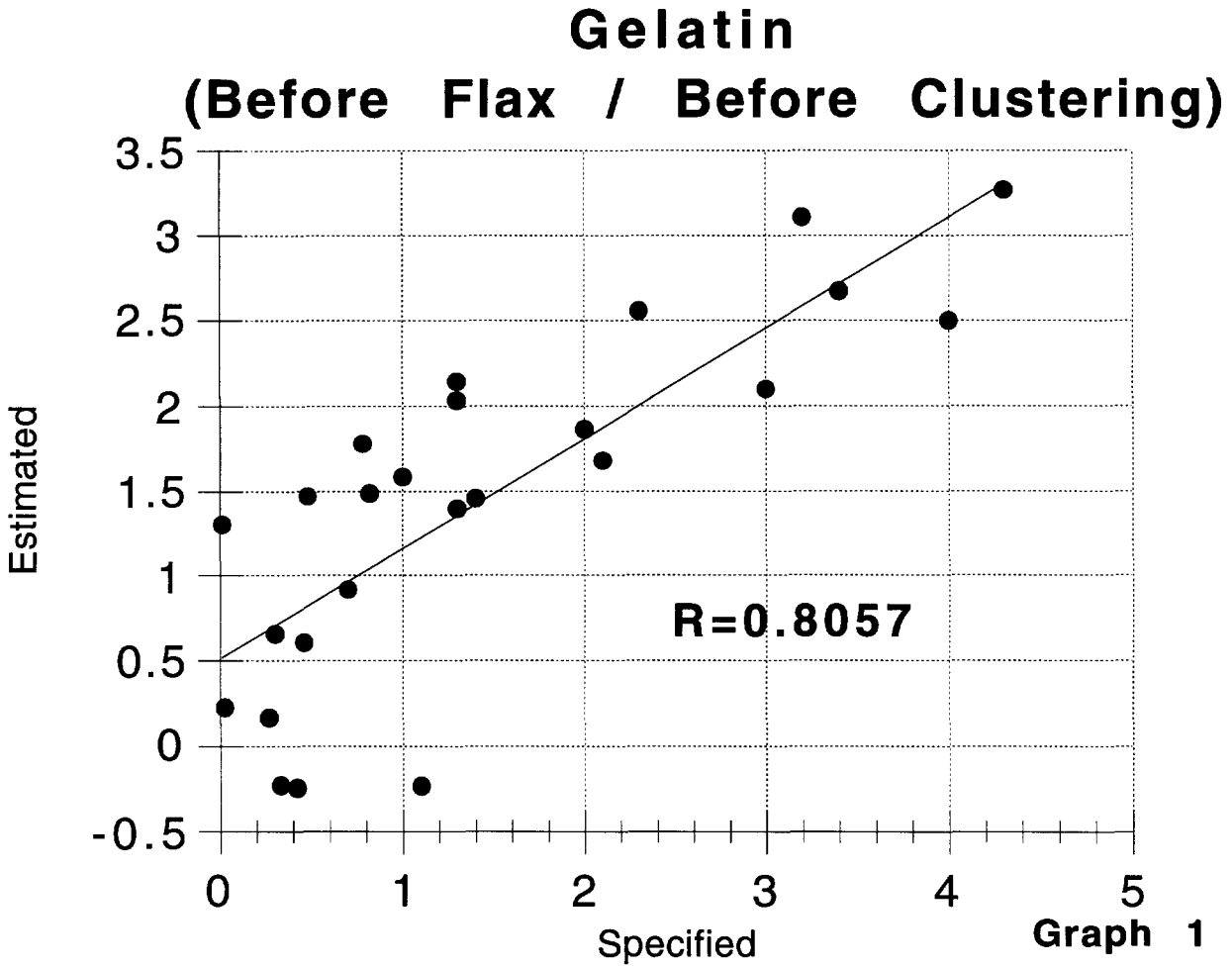

100.06
99.5
99.0
98.5
98.0
97.5
97.0
96.5
96.0
95.5
95.0
94.5
94.0
93.5
93.0
92.5
92.0
91.66
%T
AMIDE I
AMIDE II
CELLULOSE
SPECTRUM 1
4000.0
3000
2000
1500
1000
500
400.0
cm-1
a:\flaxav80.sp

Principal Components Regression



$$\text{Conc} = k_0 + k_1 \times \text{loading } 1 + \ldots + k_n \times \text{loading } n$$

Figure 2


Gelatin
(Before Flax / Before Clustering)
Estimated
Specified
R=0.8057
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
0
1
2
3
4
5

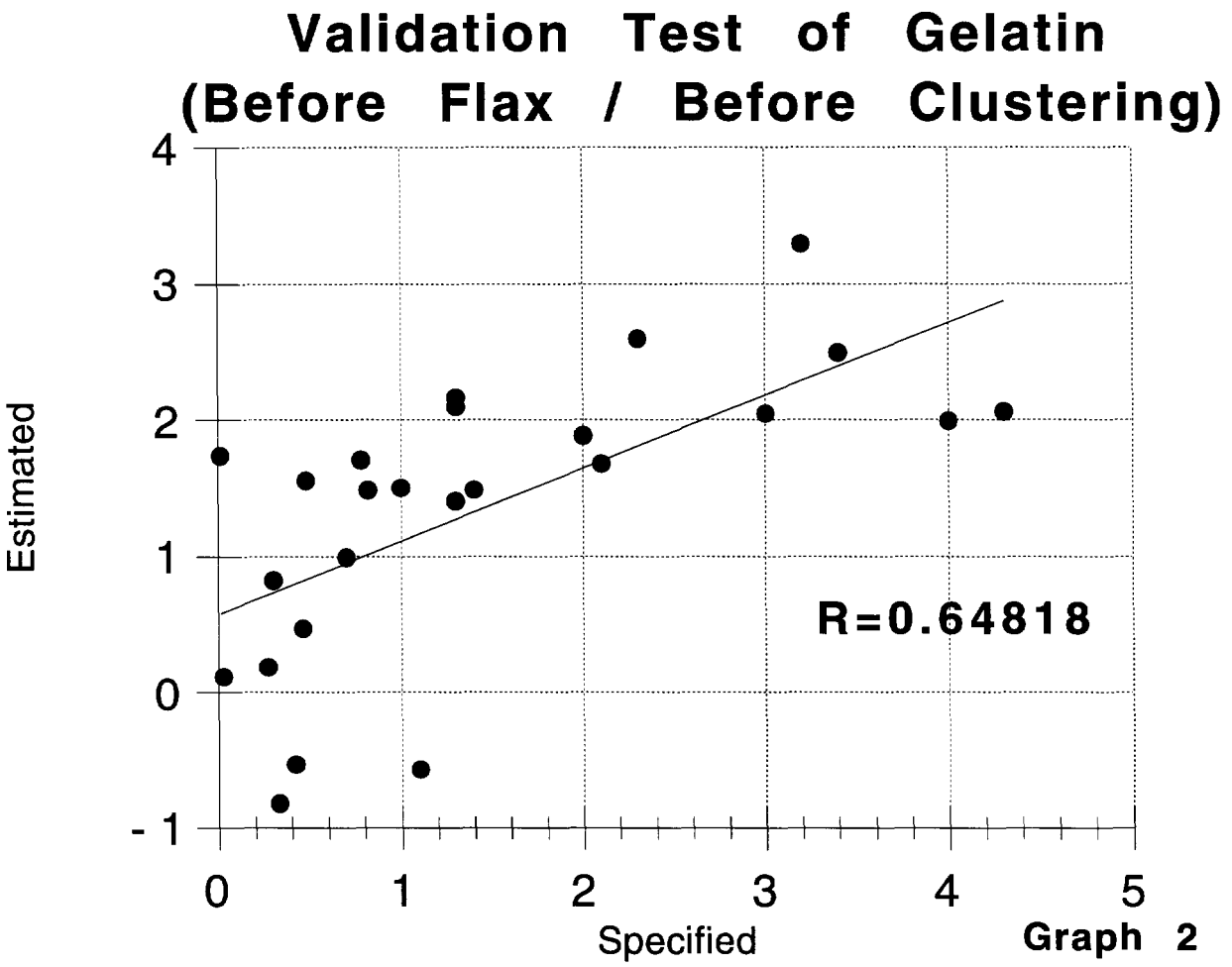
Graph 1

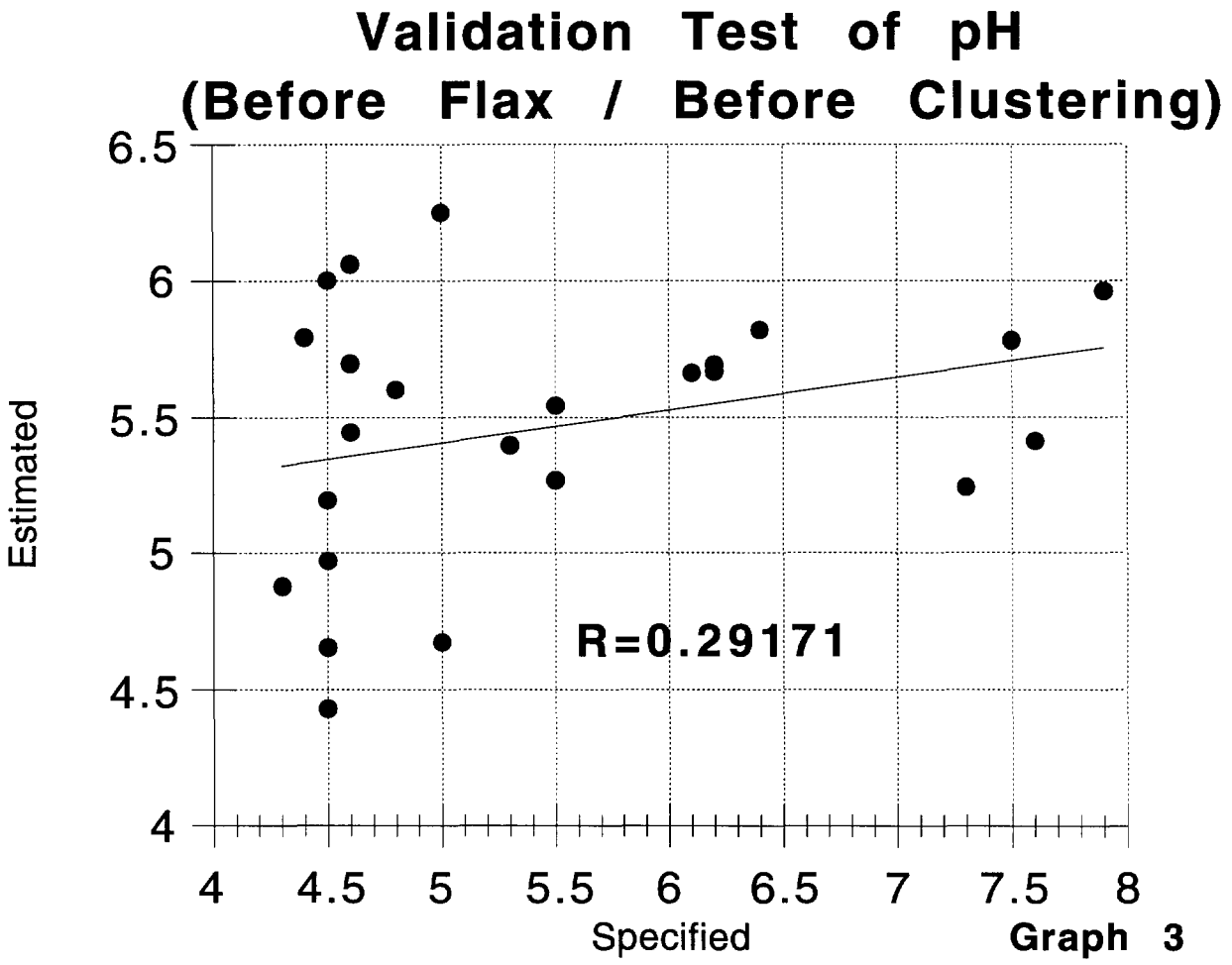
CORRELATIONS BEFORE FLAX SAMPLES WERE ADDED TO CALIBRATION

AND BEFORE CLUSTERING

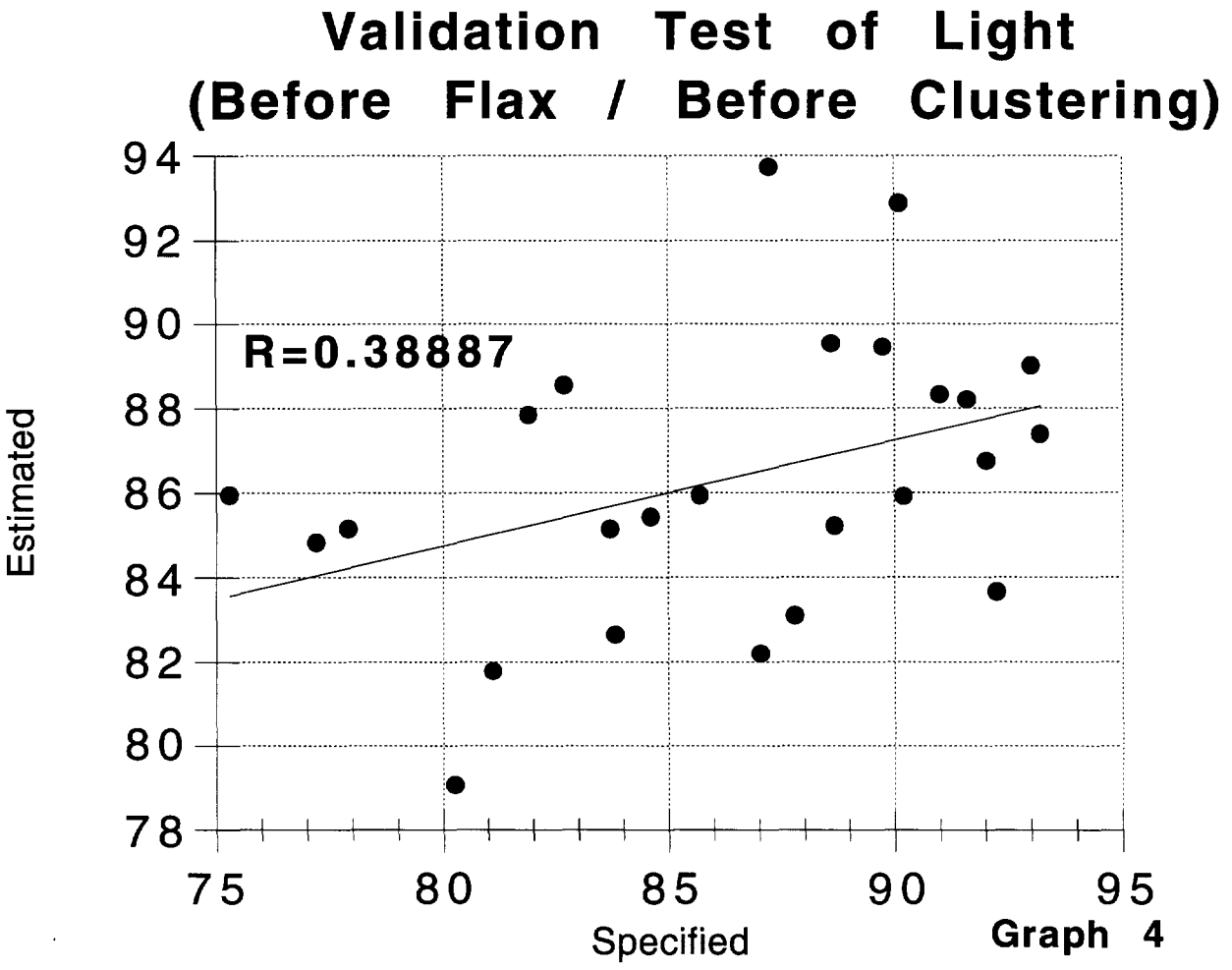
PROPERTY	PCR FACTOR	CORRELATION COEFFICIENT	MULTIPLE CORRELATION (IF APPLICABLE)
GELATIN	3 4 5	0.3325 -0.5441 0.4633	0.8057
pH	2	0.4540	
LIGHT	5	0.5396	
CALCIUM	5	0.3514	
SULFUR	1 4 5	0.1782 -0.5303 -0.2821	0.7937
POTASSIUM	1 4 5	0.2654 -0.5882 0.1492	0.8284
IRON	2 4	0.4186 0.4113	0.5735
FLUORESCENCE	5	-0.4495	
DATE	3	-0.6882	

Table 1

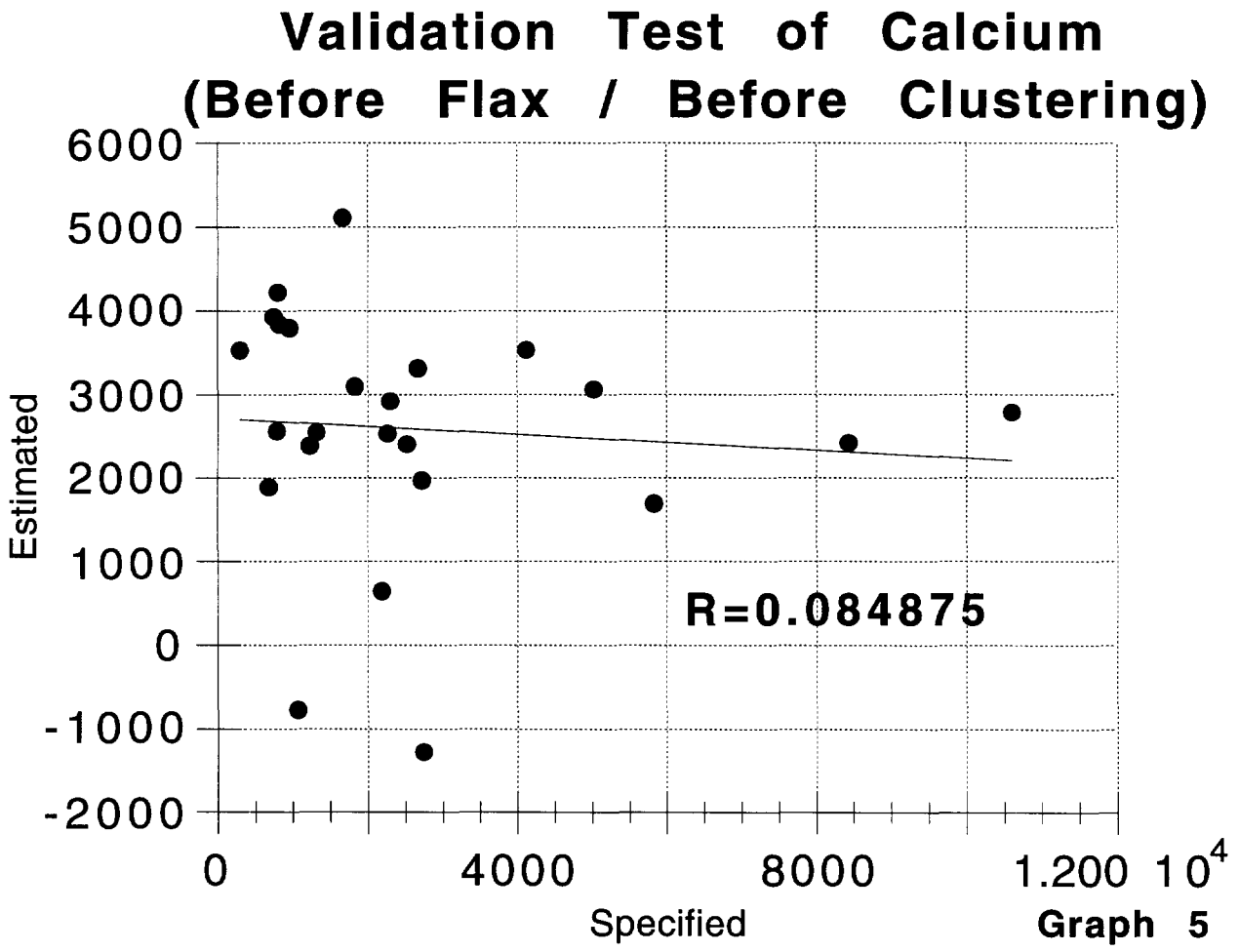

Validation Test of Gelatin
(Before Flax / Before Clustering)
Estimated
Specified
R=0.64818
4
3
2
1
0
- 1
0
1
2
3
4
5
Graph 2


Validation Test of pH
(Before Flax / Before Clustering)
Estimated
6.5
6
5.5
5
4.5
4
R=0.29171
4
4.5
5
5.5
6
6.5
7
7.5
8
Specified

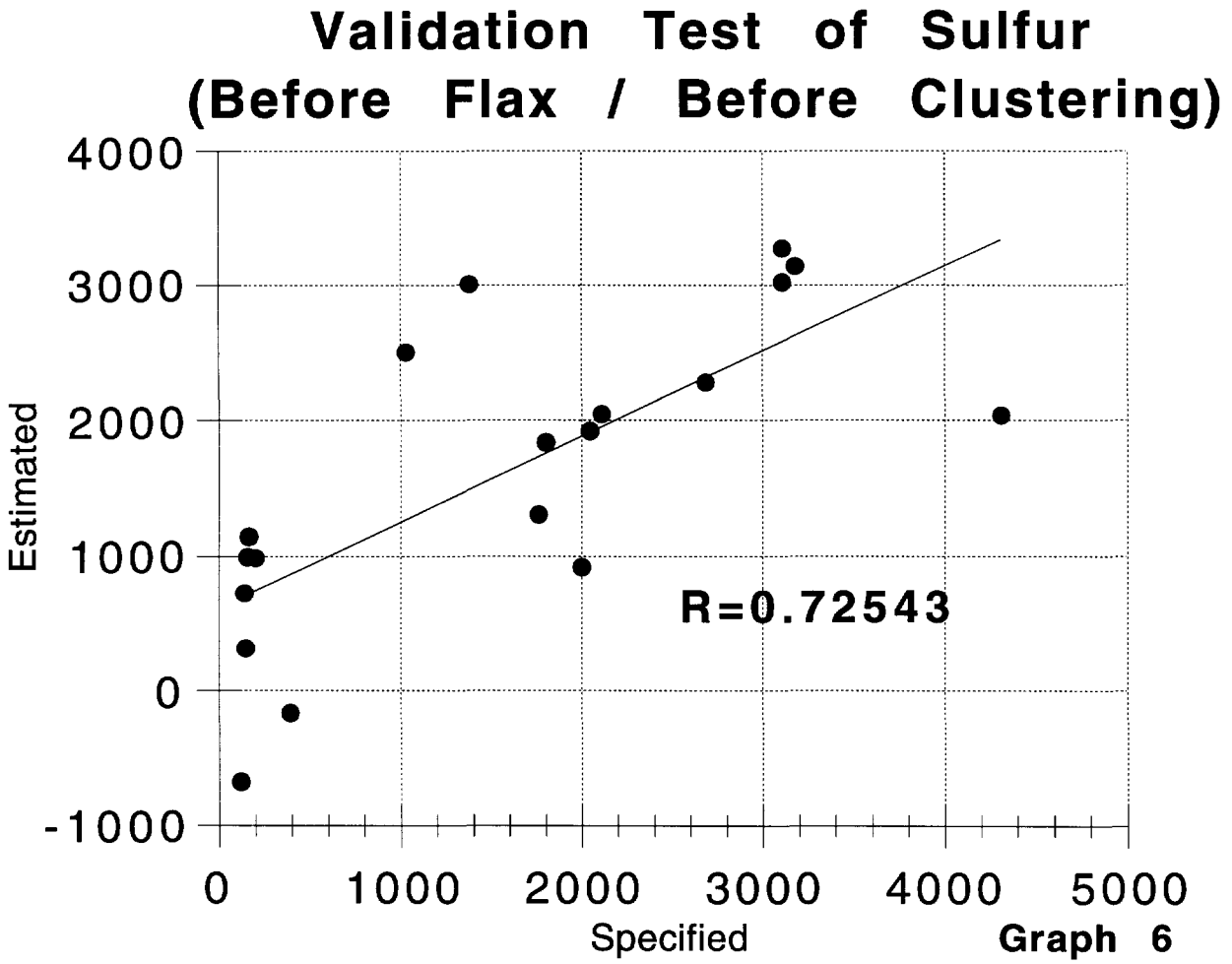
Graph 3


Validation Test of Light
(Before Flax / Before Clustering)
R=0.38887
Estimated
Specified
94
92
90
88
86
84
82
80
78
75
80
85
90
95

Graph 4

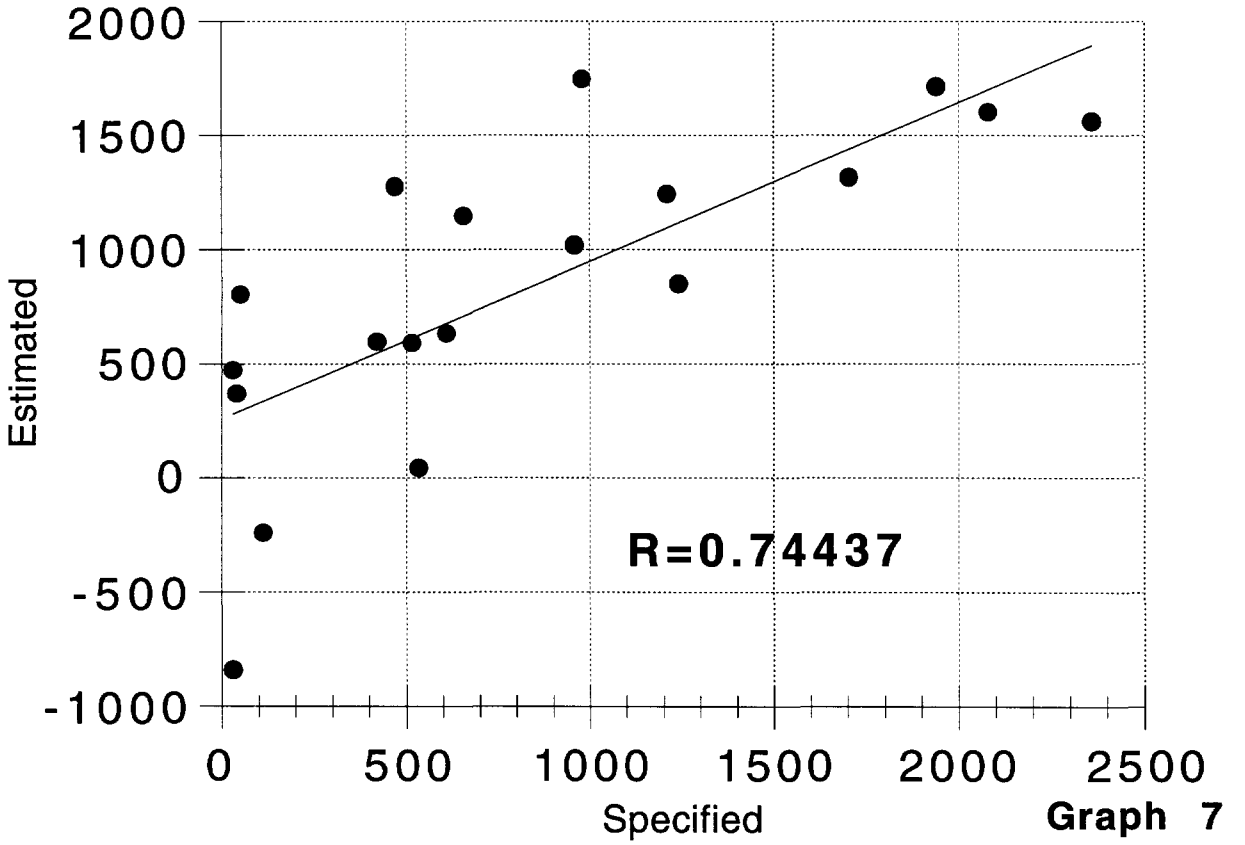

Validation Test of Calcium
(Before Flax / Before Clustering)
6000
5000
4000
3000
2000
1000
0
-1000
-2000
Estimated
R=0.084875
0
4000
8000
1.200 10^4
Specified

Graph 5

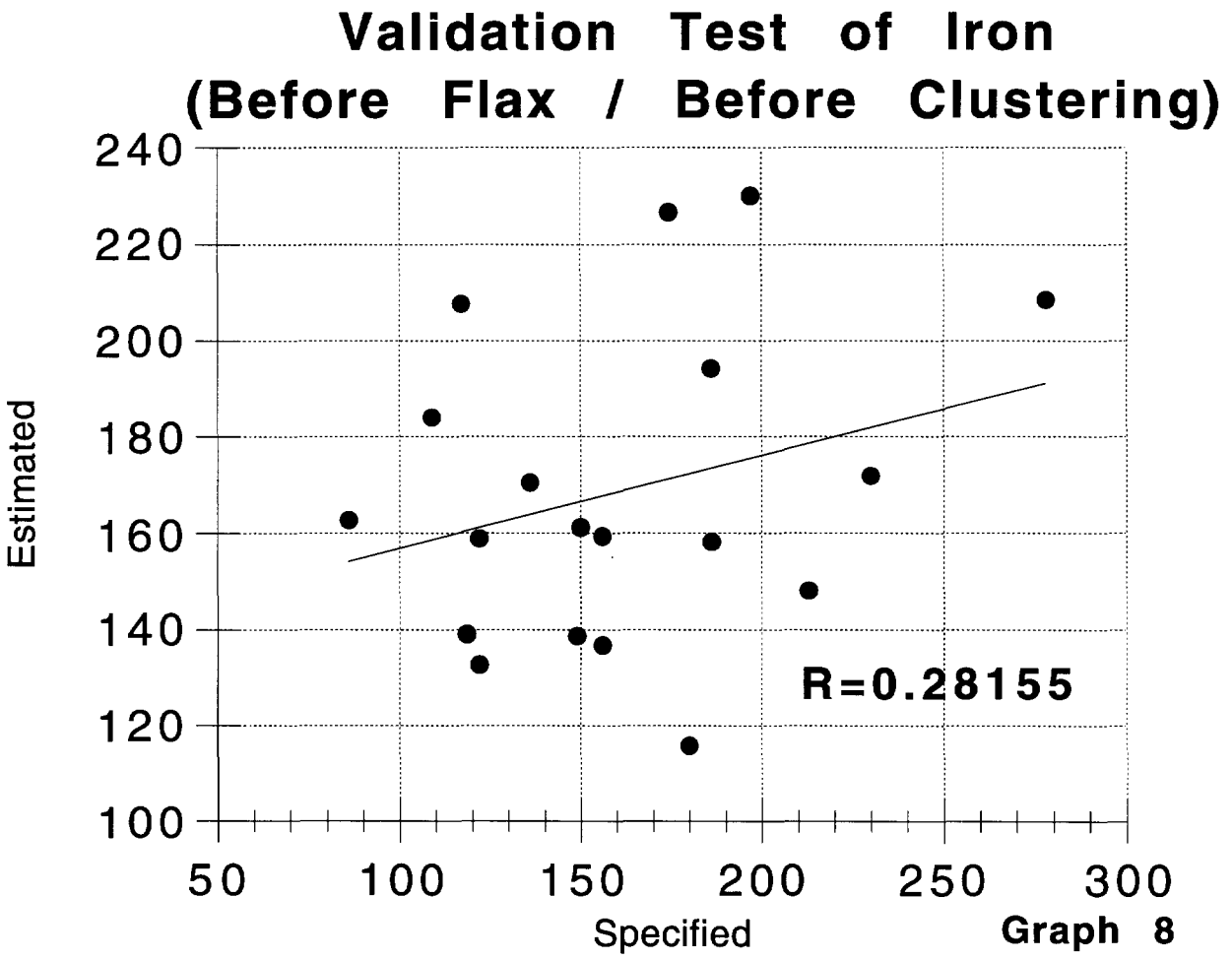

Validation Test of Sulfur
(Before Flax / Before Clustering)
4000
3000
2000
1000
0
-1000
Estimated
R=0.72543
0
1000
2000
3000
4000
5000
Specified

Graph 6

Validation Test of Potassium (Before Flax / Before Clustering)

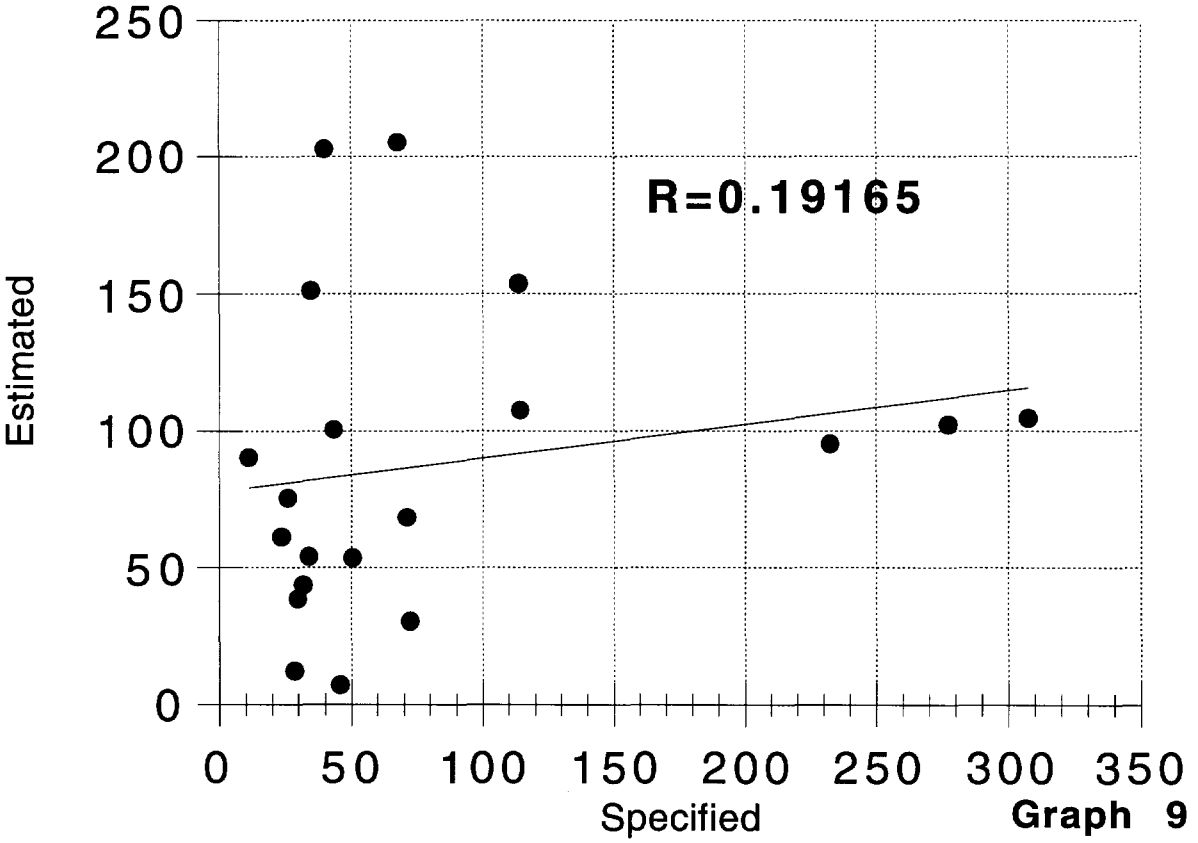


Graph 7

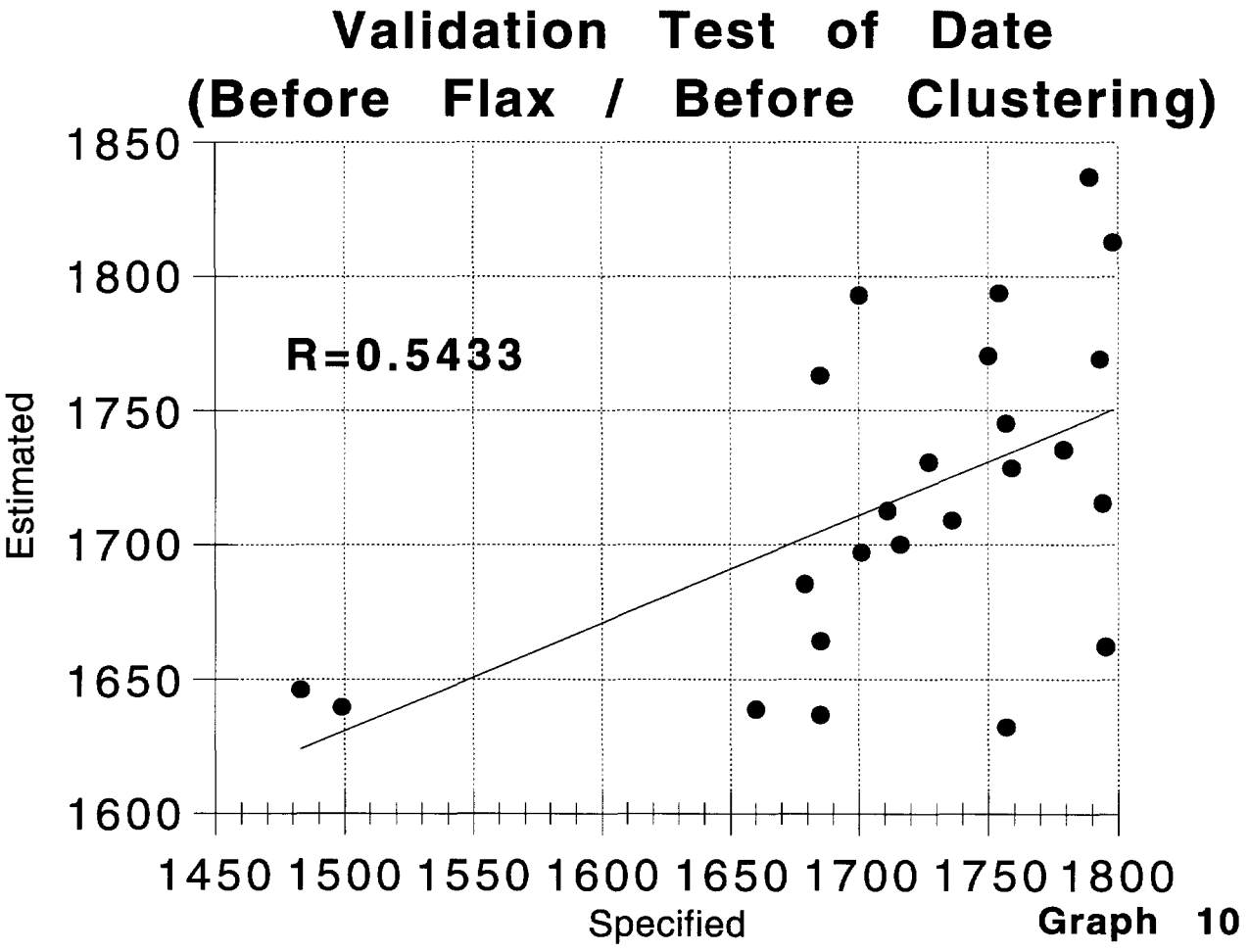

Validation Test of Iron
(Before Flax / Before Clustering)
240
220
200
180
160
140
120
100
Estimated
50
100
150
200
250
300
Specified
R=0.28155

Graph 8

Validation Test of Fluorescence (Before Flax / Before Clustering)



Graph 9


Validation Test of Date
(Before Flax / Before Clustering)
R=0.5433
Estimated
1850
1800
1750
1700
1650
1600
1450 1500 1550 1600 1650 1700 1750 1800
Specified

Graph 10

IR PREDICTED PROPERTIES OF ARTIFICIALLY AGED FILTER PAPERS

SAMPLE	PREDICTED % GELATIN	PREDICTED L*	PREDICTED pH	PREDICTED DATE	PREDICTED CALCIUM
PAPER WITH NO GELATIN	.4	73.6	5.8	1663	-496.5
PAPER WITH GELATIN	2.5	85.9	5.4	1646	2623
PAPER WITH GELATIN, SIZED	2.0	83.2	5.5	1676	1928

Table 2

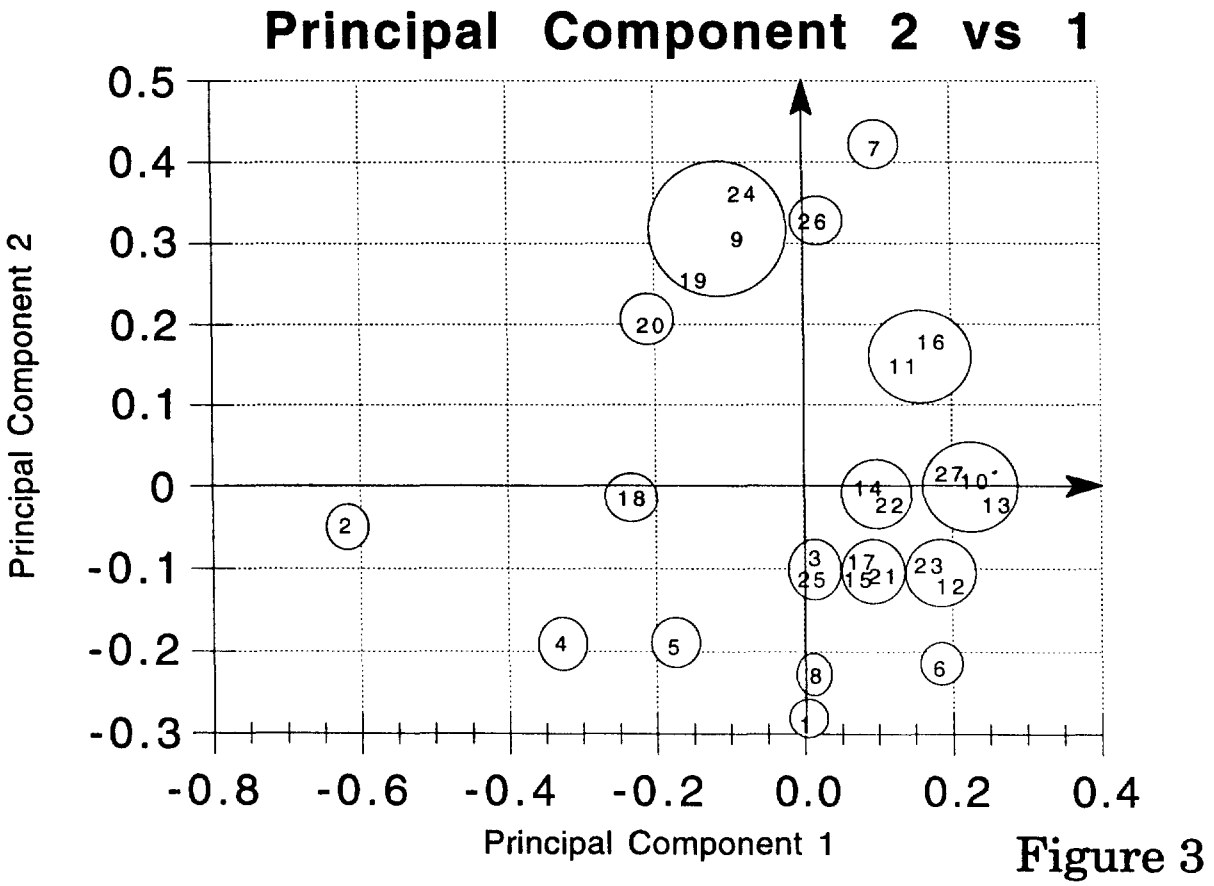
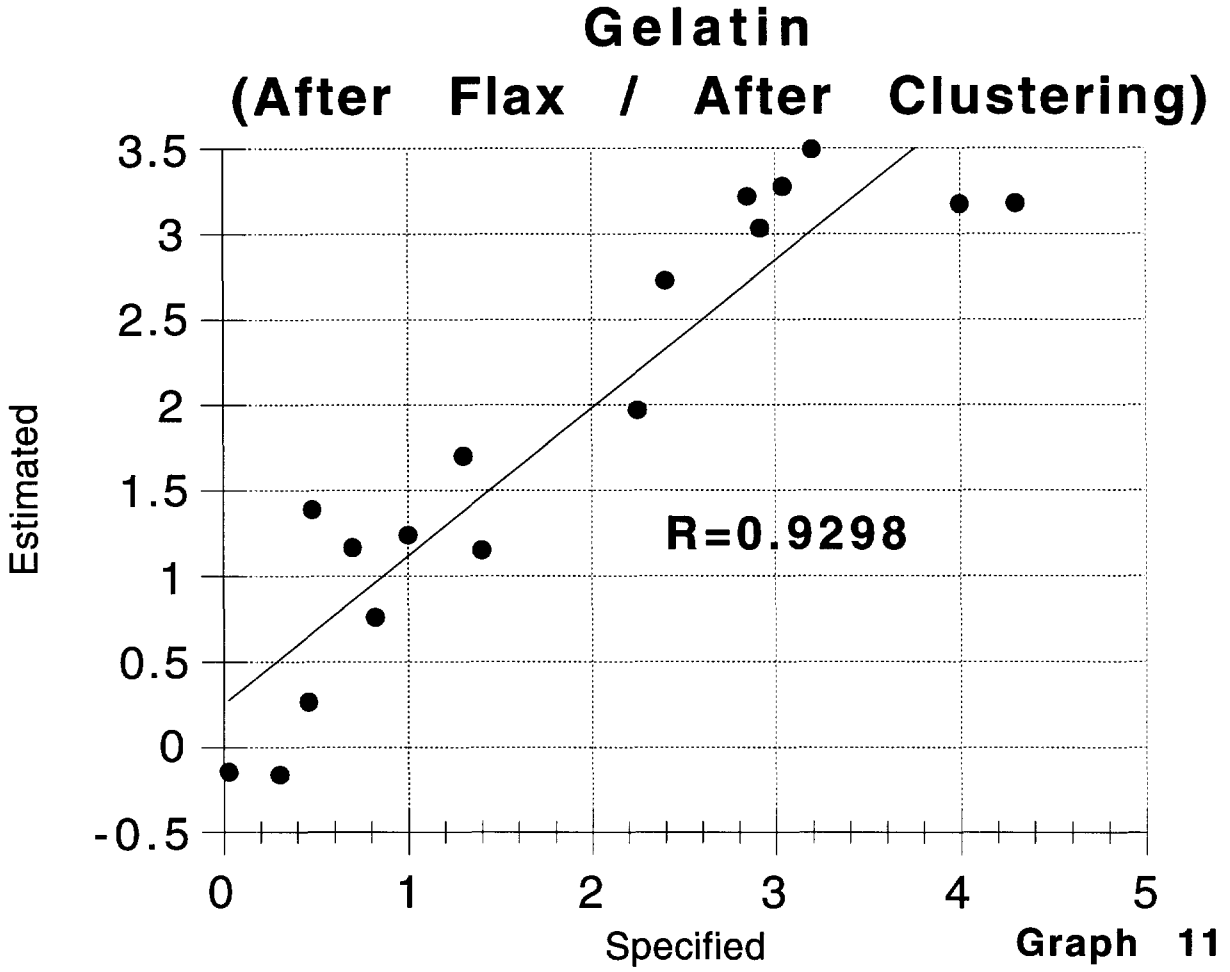

Principal Component 2 vs 1
Principal Component 2
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.8
-0.6
-0.4
-0.2
0.0
0.2
0.4
Principal Component 1
7
24
9
19
26
20
16
11
18
14
22
27
10
13
2
3
25
17
15
21
23
12
4
5
8
6
1

Figure 3

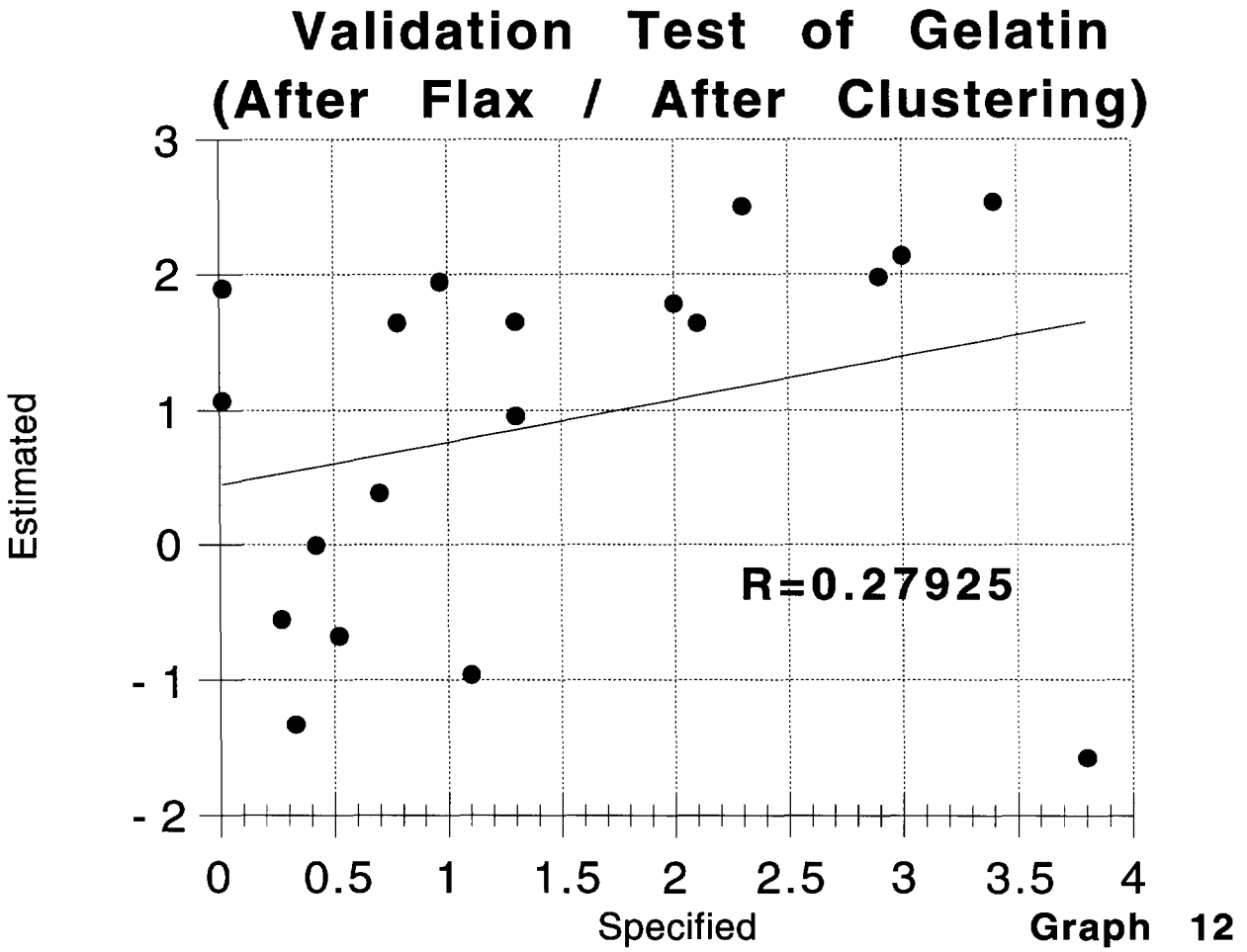

Gelatin
(After Flax / After Clustering)
Estimated
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
0
1
2
3
4
5
Specified
R=0.9298

Graph 11

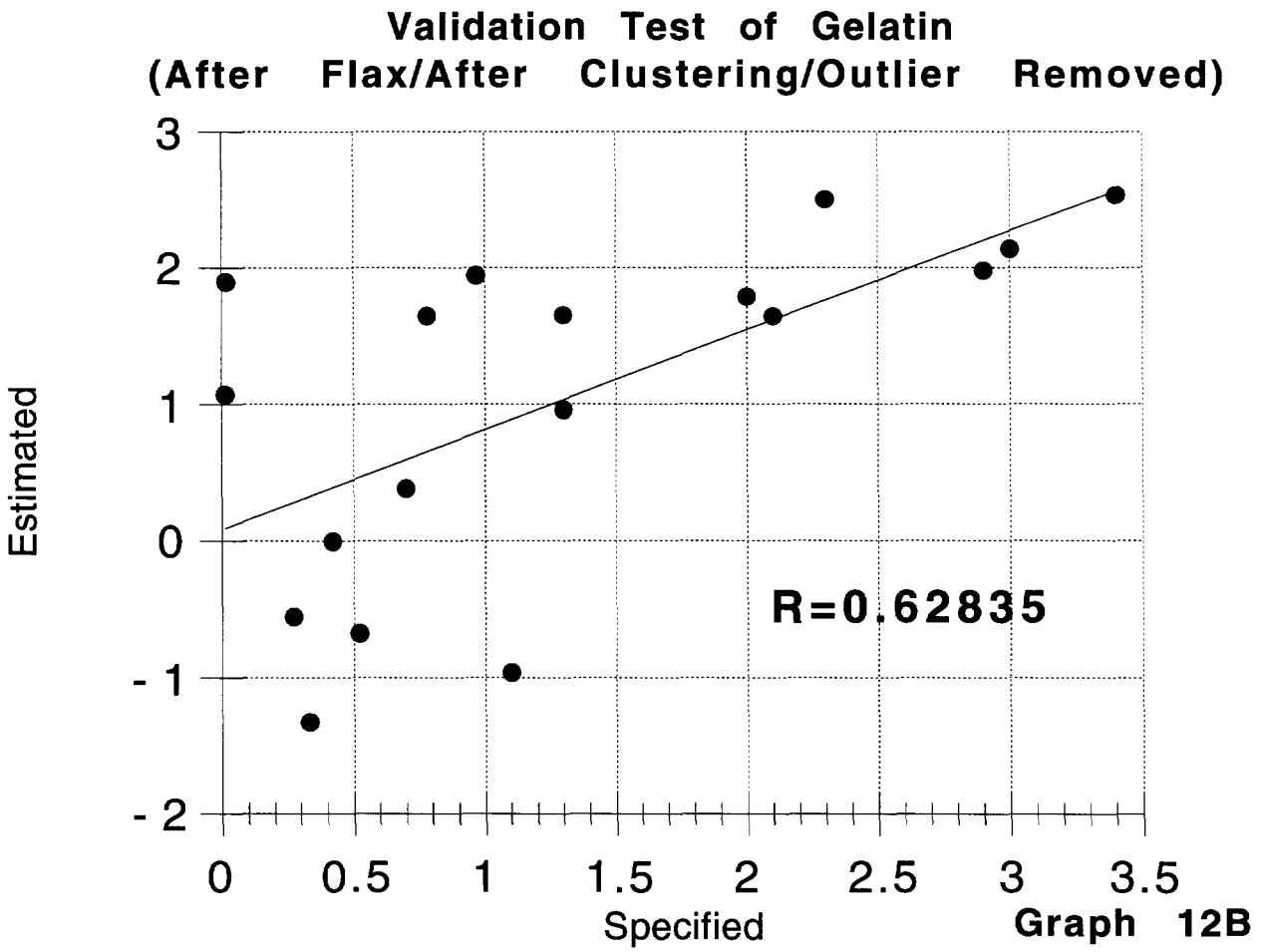
CORRELATIONS AFTER FLAX SAMPLES WERE ADDED TO CALIBRATION AND AFTER CLUSTERING

PROPERTY	PCR FACTOR	CORRELATION COEFFICIENT	MULTIPLE CORRELATION (IF APPLICABLE)
GELATIN	1 3 4 5	-0.3132 -0.4562 0.5464 0.5096	0.9298
pH	4	-0.4560	
LIGHT	5	0.6020	
CALCIUM	5	0.7227	
SULFUR	3 4	0.5105 0.4919	0.8665
POTASSIUM	4	-0.5391	
IRON	1 2 4 5	-0.0409 -0.9937 -0.1925 -0.0378	0.9985
FLUORESCENCE	2 4 5	0.8262 0.3358 0.3825	0.9687
DATE	1 2 3	0.4376 -0.2422 0.4407	0.8165

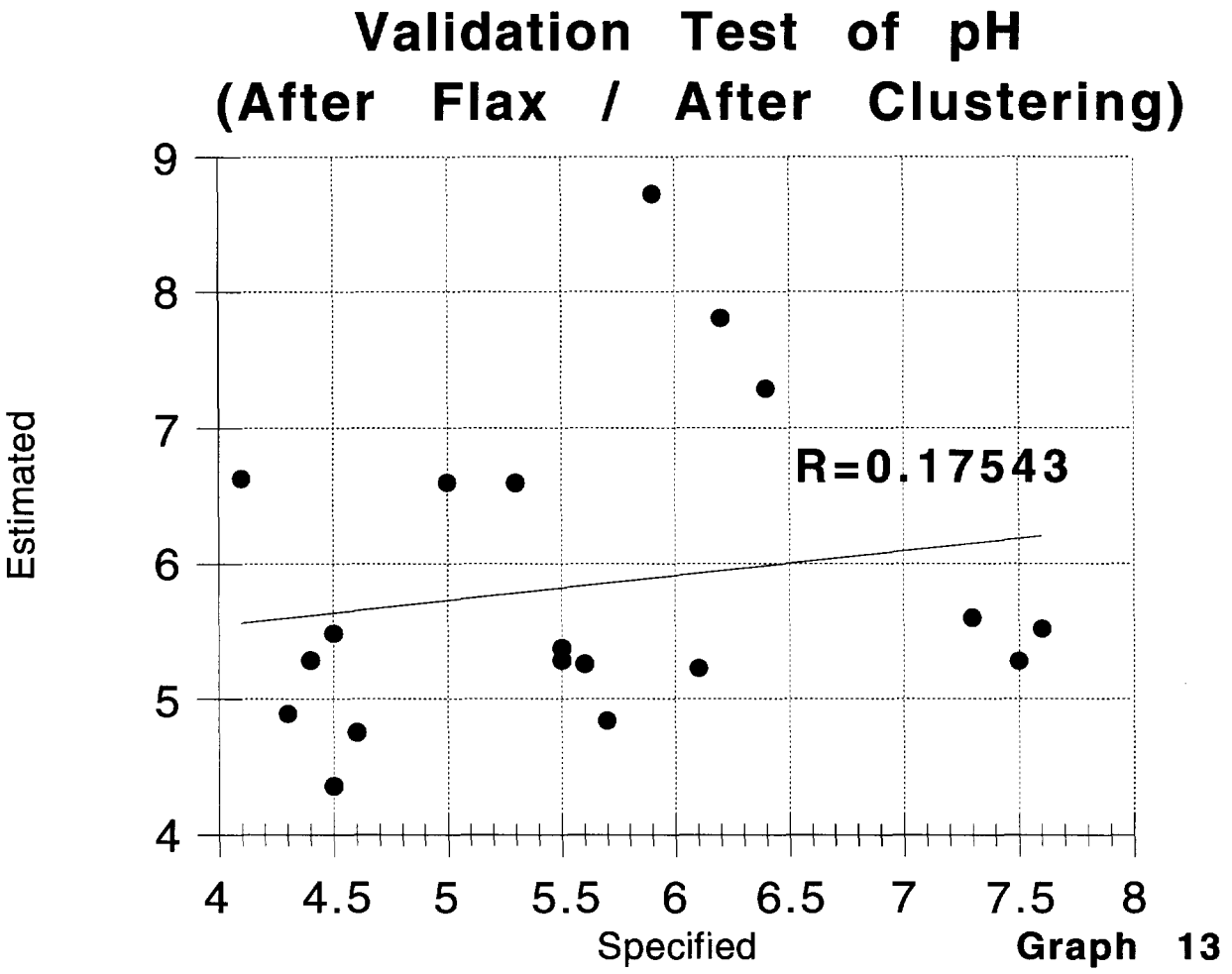
Table 3


Validation Test of Gelatin
(After Flax / After Clustering)
Estimated
3
2
1
0
- 1
- 2
R=0.27925
0
0.5
1
1.5
2
2.5
3
3.5
4
Specified

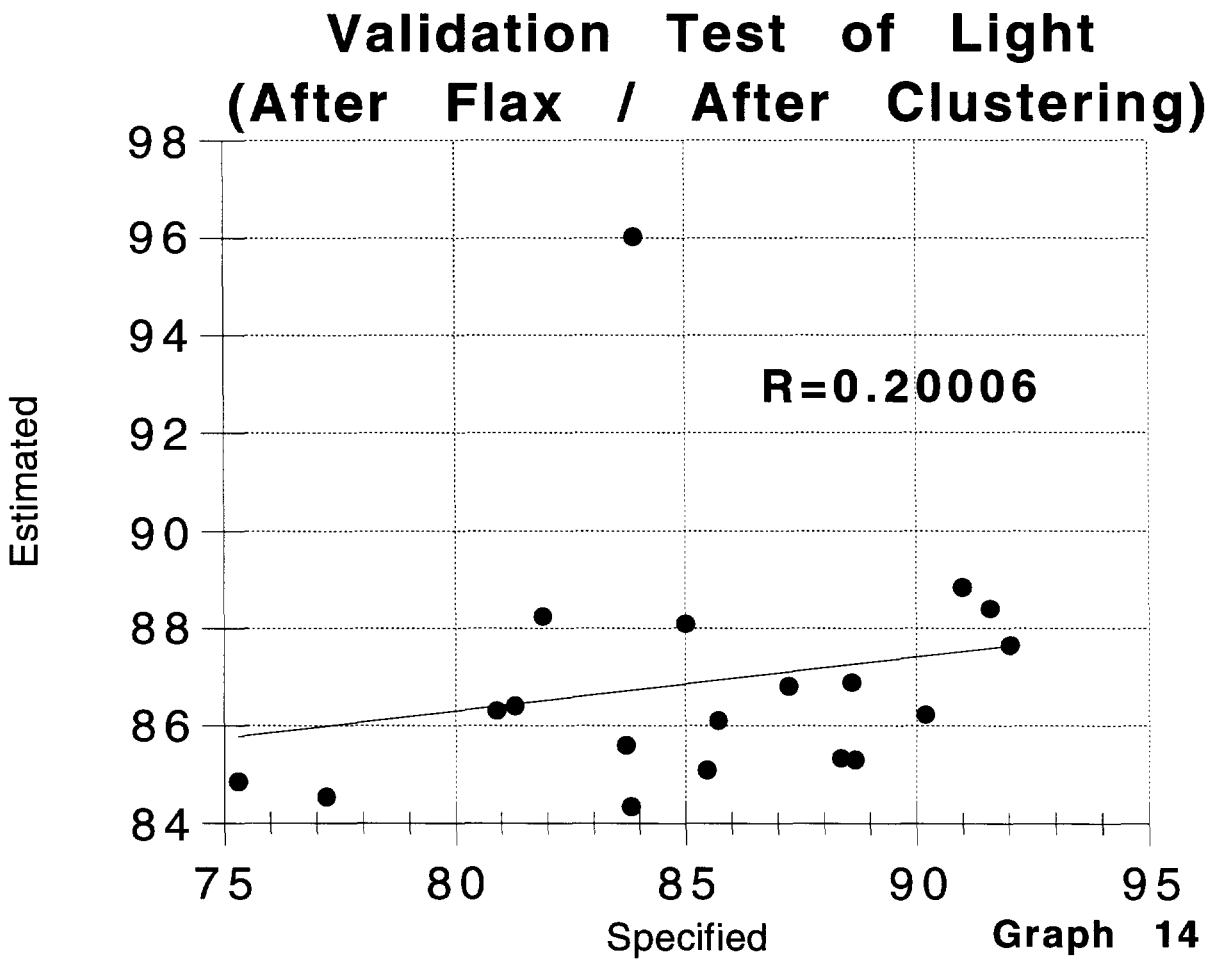
Graph 12


Validation Test of Gelatin
(After Flax/After Clustering/Outlier Removed)
Estimated
3
2
1
0
- 1
- 2
0
0.5
1
1.5
2
2.5
3
3.5
Specified
R=0.62835

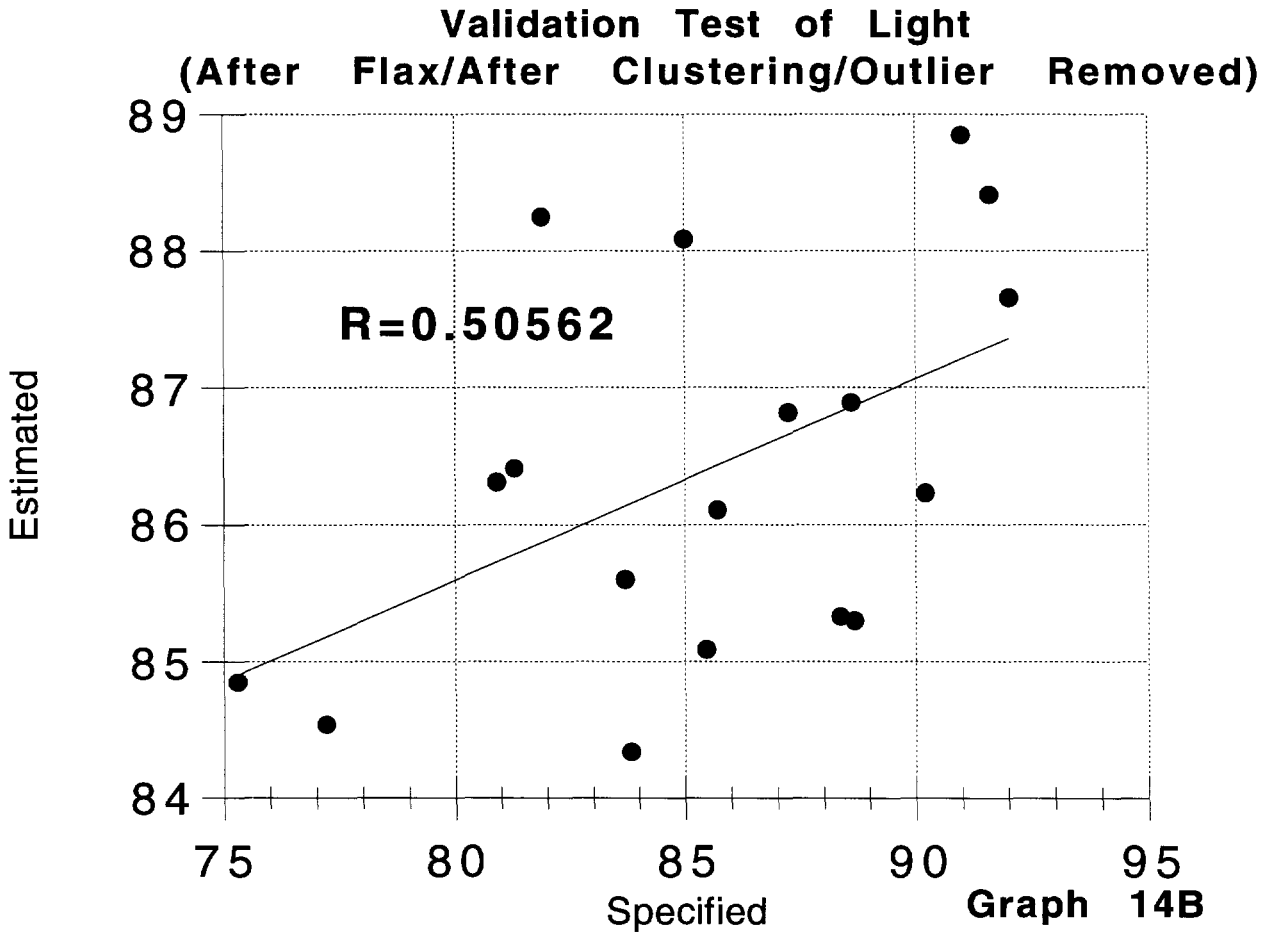
Graph 12B


Validation Test of pH
(After Flax / After Clustering)
Estimated
Specified
R=0.17543
9
8
7
6
5
4
4
4.5
5
5.5
6
6.5
7
7.5
8

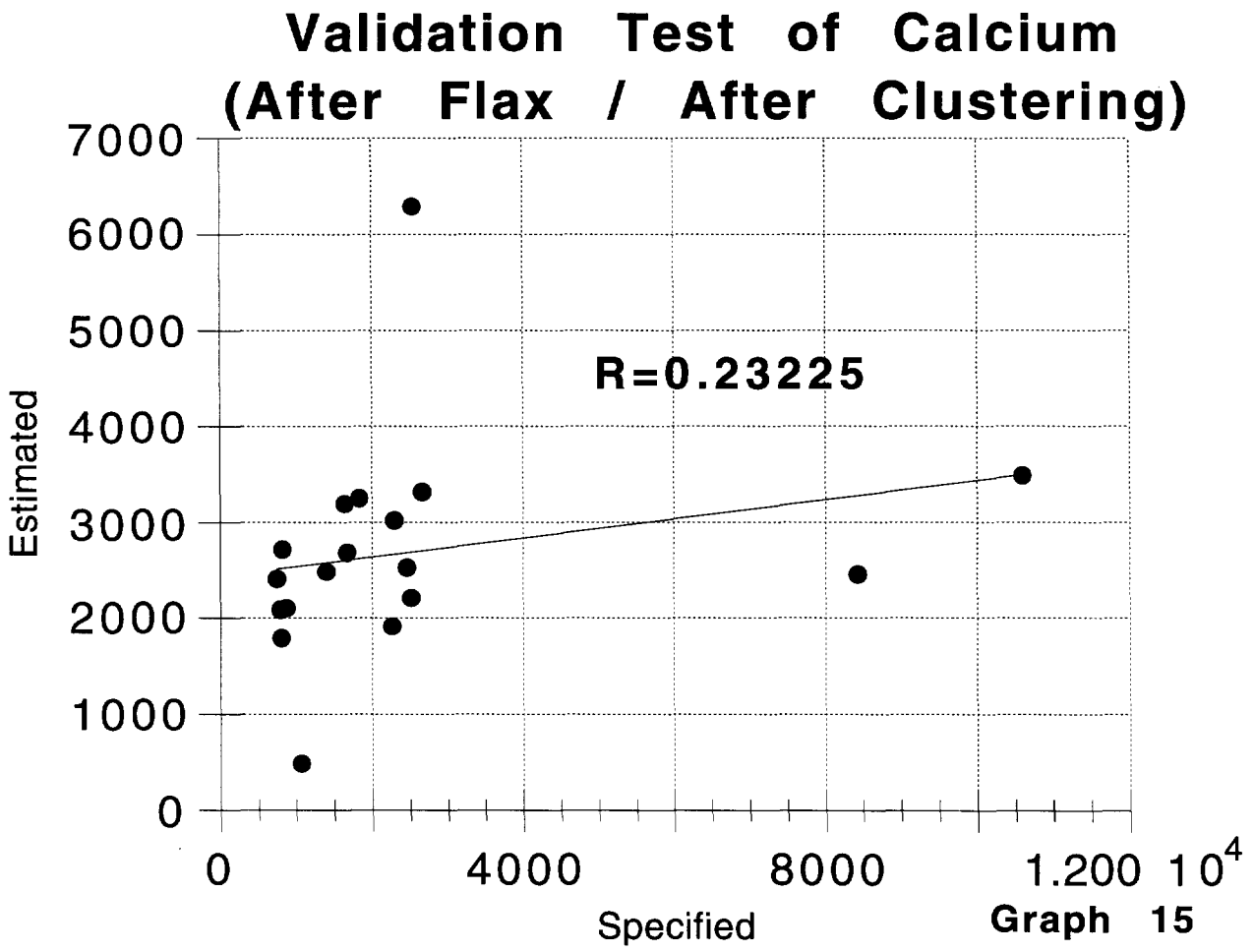
Graph 13


Validation Test of Light
(After Flax / After Clustering)
98
96
94
92
90
88
86
84
Estimated
R=0.20006
75
80
85
90
95
Specified

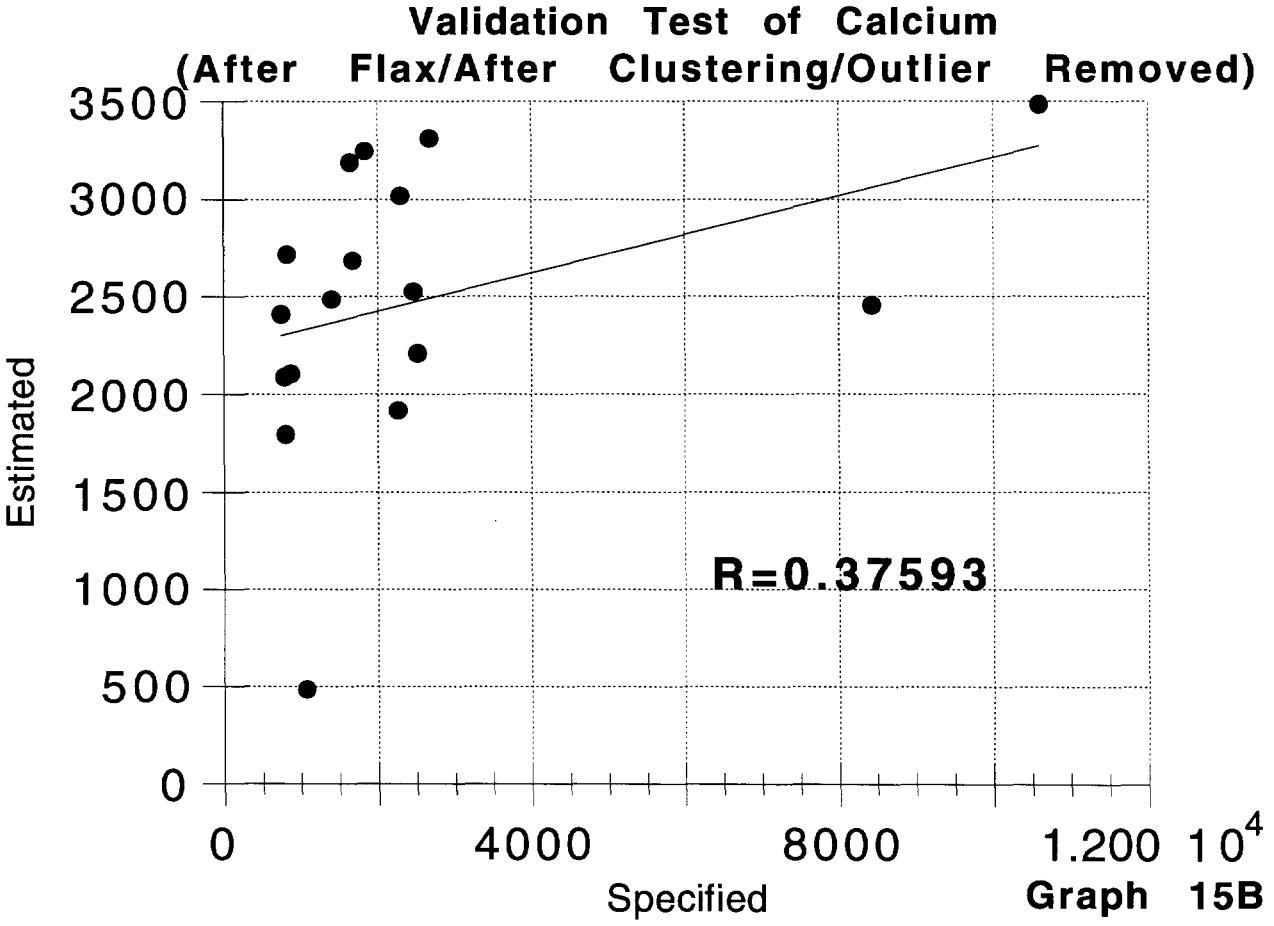
Graph 14


Validation Test of Light
(After Flax/After Clustering/Outlier Removed)
R=0.50562
Estimated
89
88
87
86
85
84
75
80
85
90
95
Specified

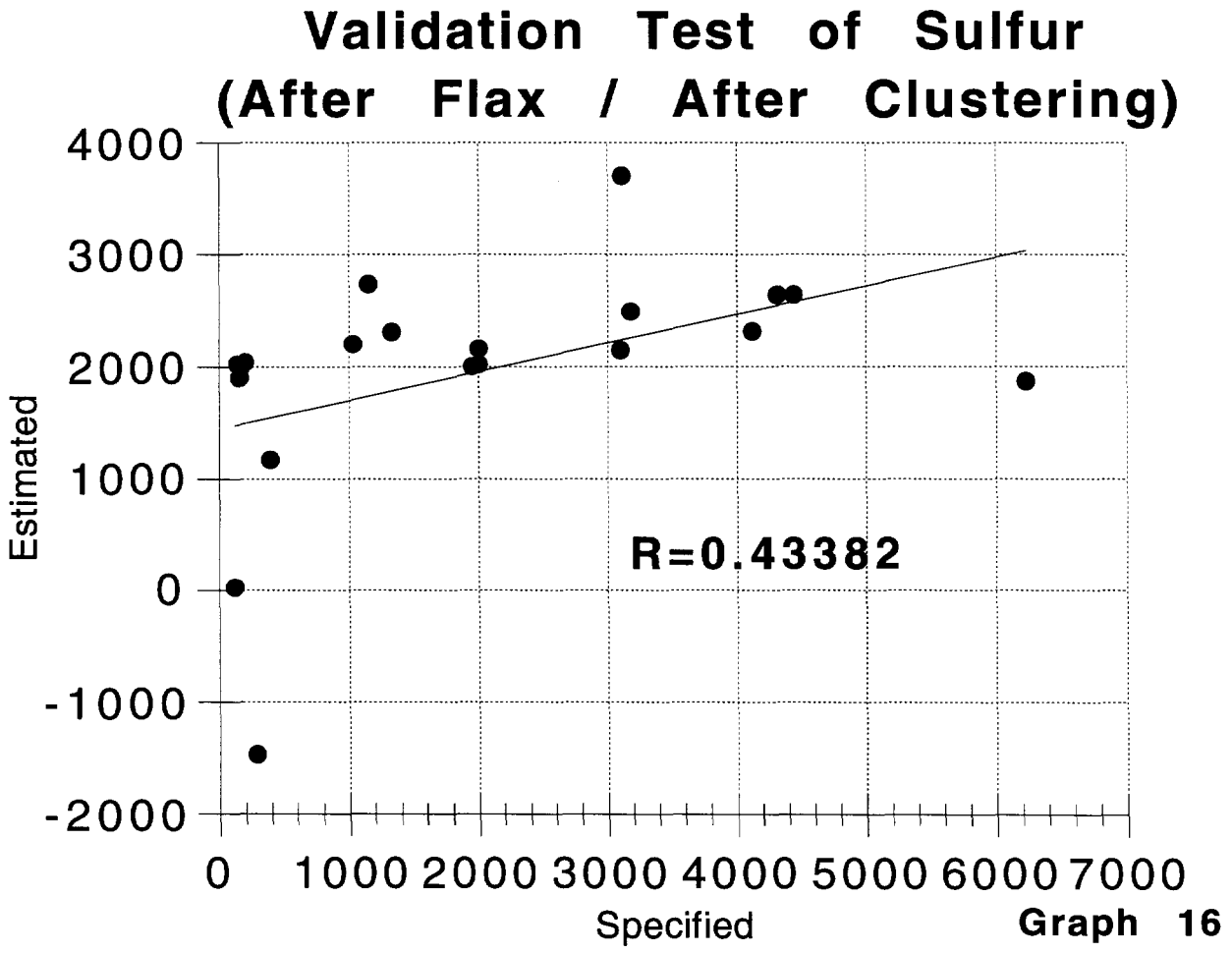
Graph 14B


Validation Test of Calcium
(After Flax / After Clustering)
7000
6000
5000
4000
3000
2000
1000
0
Estimated
R=0.23225
0
4000
8000
1.200 10^4
Specified

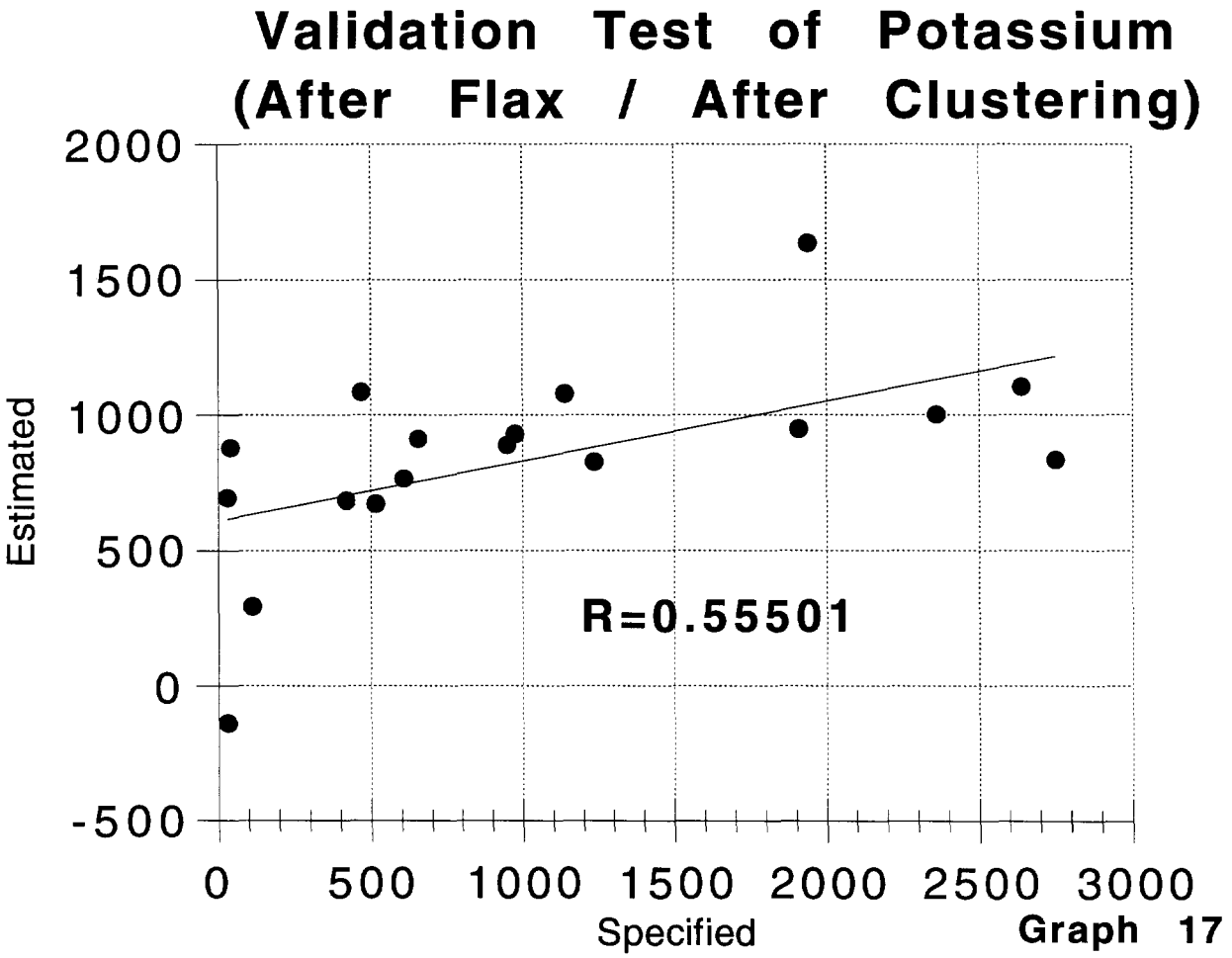
Graph 15


Validation Test of Calcium
(After Flax/After Clustering/Outlier Removed)
Estimated
3500
3000
2500
2000
1500
1000
500
0
R=0.37593
0
4000
8000
1.200 10^4
Specified

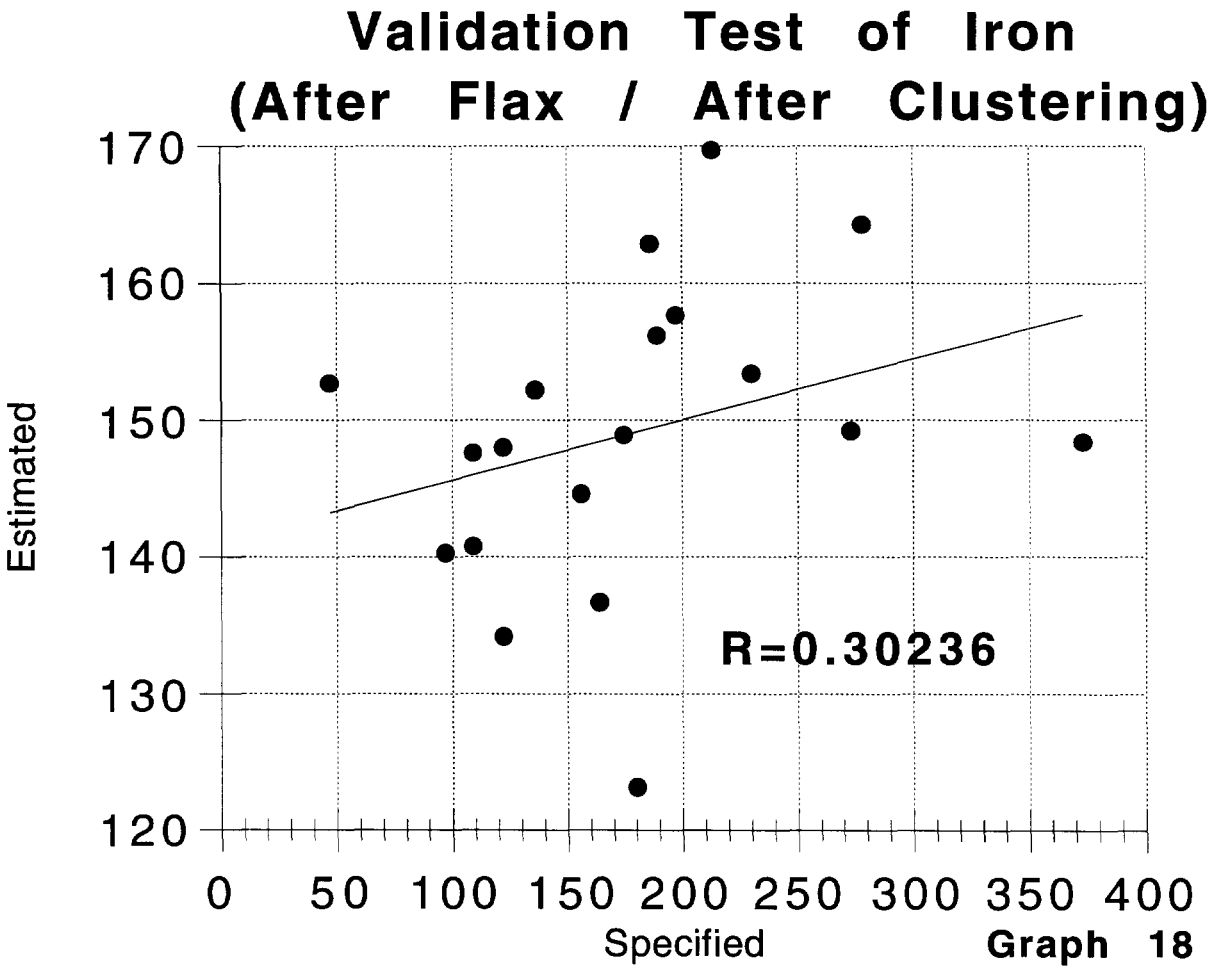
Graph 15B


Validation Test of Sulfur
(After Flax / After Clustering)
4000
3000
2000
1000
0
-1000
-2000
Estimated
R=0.43382
0
1000
2000
3000
4000
5000
6000
7000
Specified

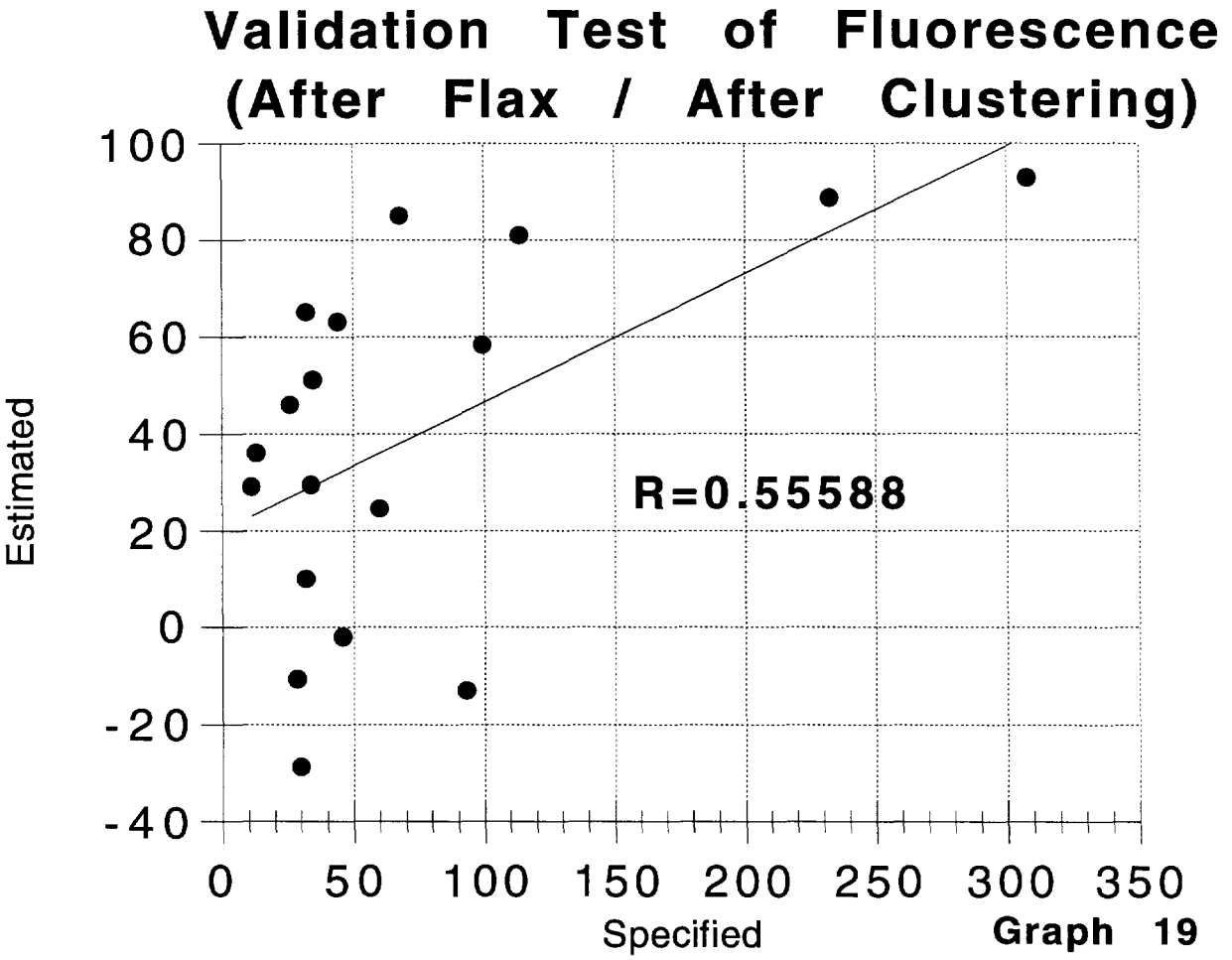
Graph 16


Validation Test of Potassium
(After Flax / After Clustering)
2000
1500
1000
500
0
-500
Estimated
R=0.55501
0
500
1000
1500
2000
2500
3000
Specified

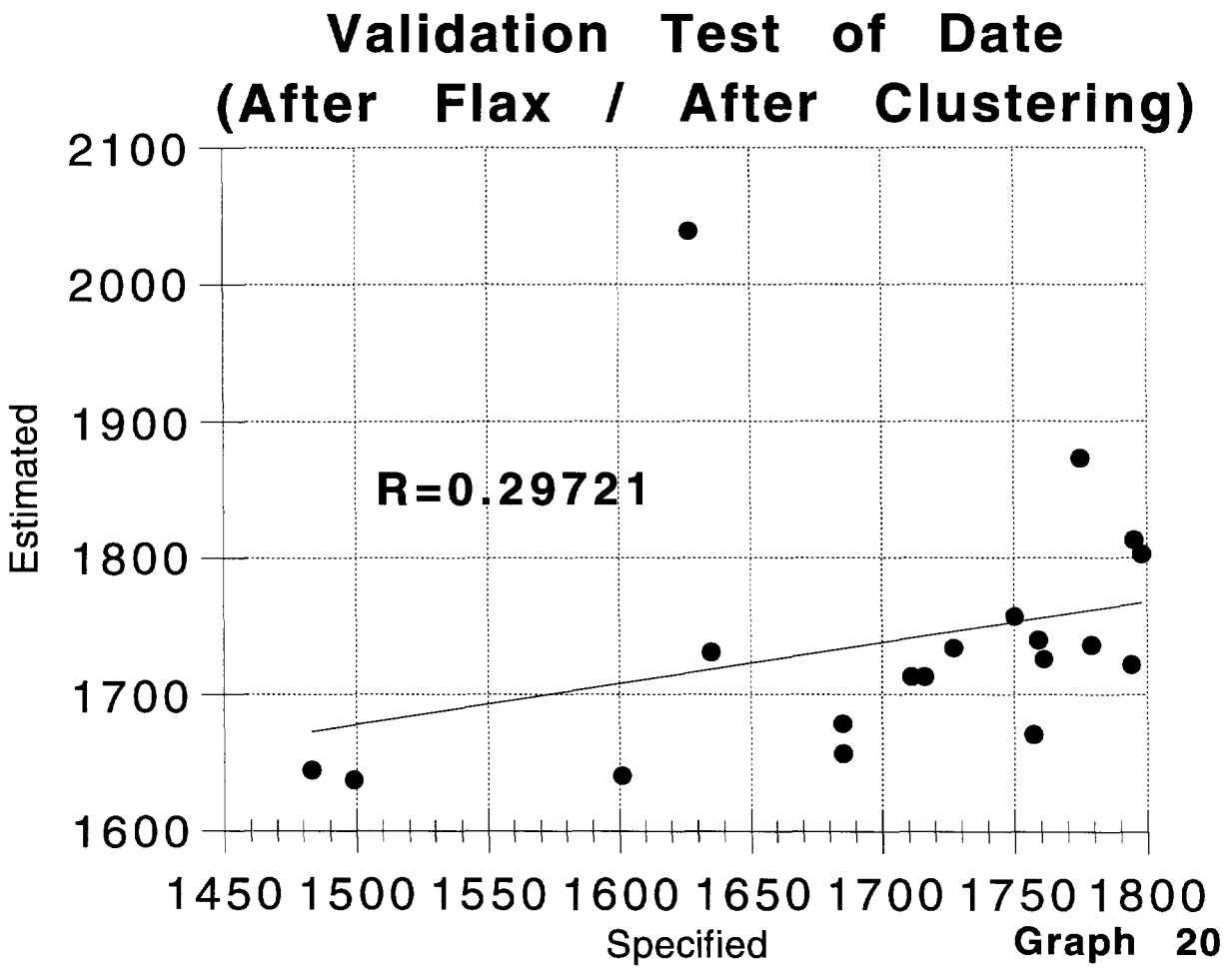
Graph 17


Validation Test of Iron
(After Flax / After Clustering)
170
160
150
140
130
120
Estimated
R=0.30236
0
50
100
150
200
250
300
350
400
Specified

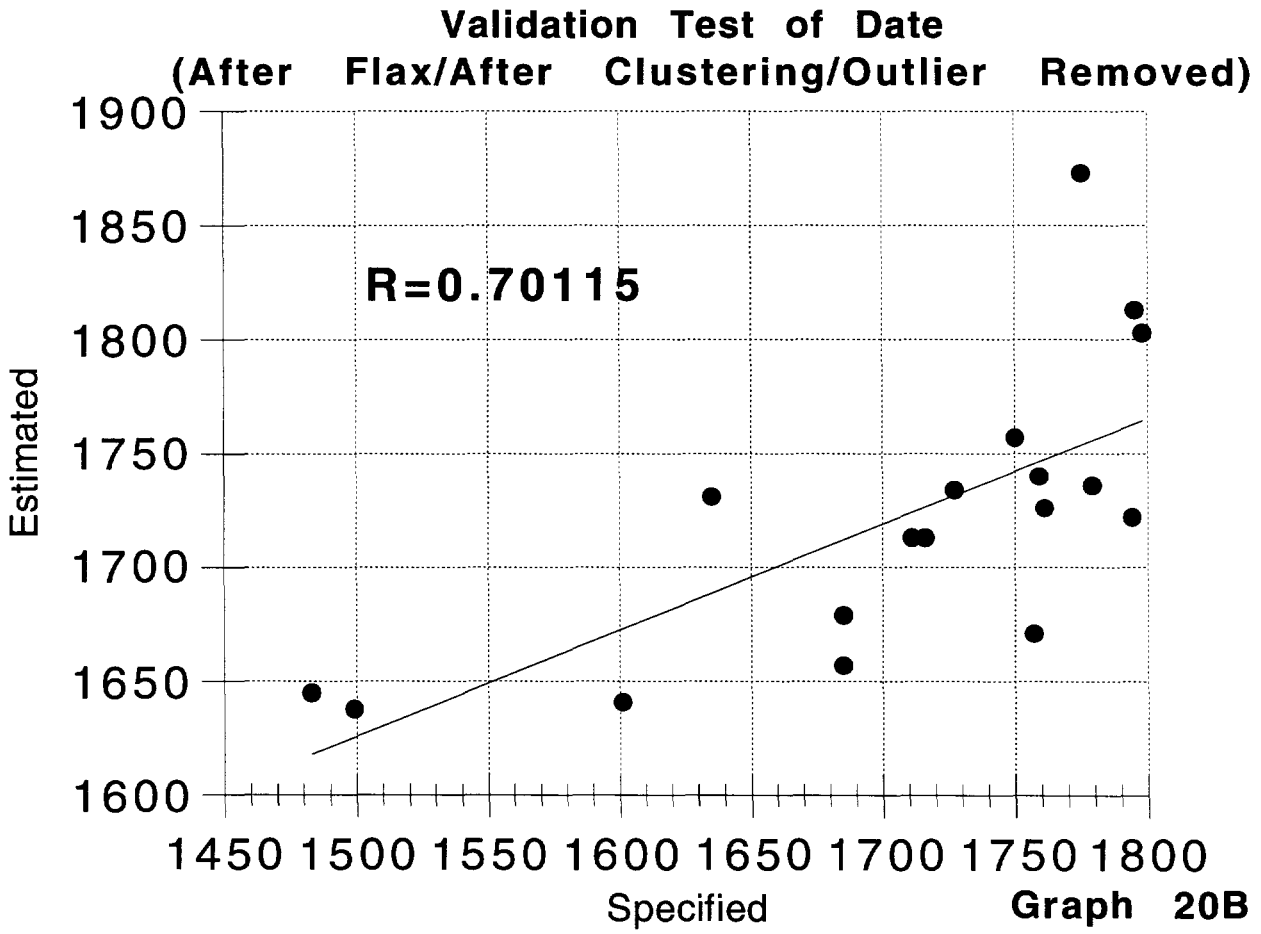
Graph 18


Validation Test of Fluorescence
(After Flax / After Clustering)
Estimated
100
80
60
40
20
0
-20
-40
R=0.55588
0
50
100
150
200
250
300
350
Specified

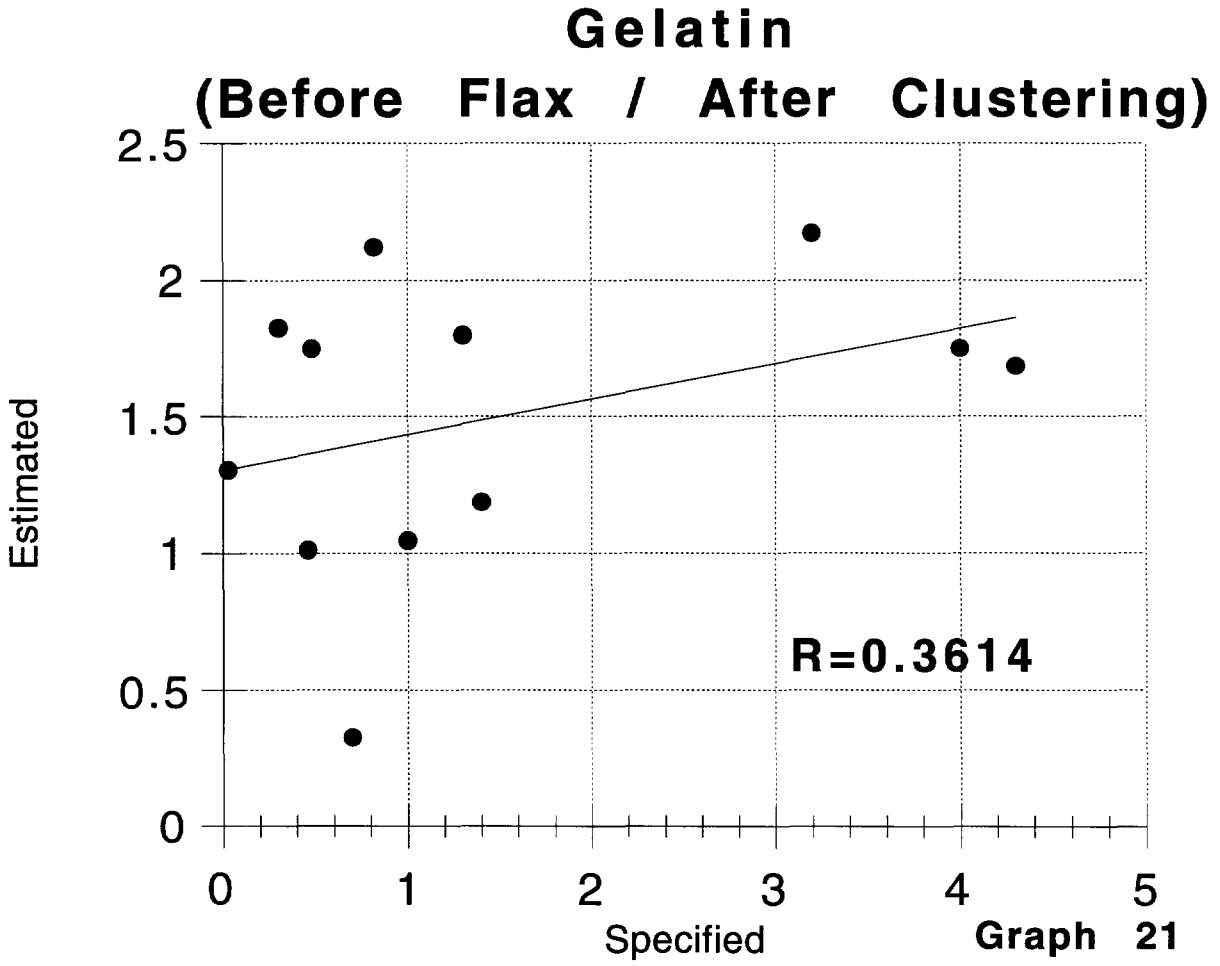
Graph 19


Validation Test of Date
(After Flax / After Clustering)
2100
2000
1900
1800
1700
1600
Estimated
R=0.29721
1450 1500 1550 1600 1650 1700 1750 1800
Specified

Graph 20


Validation Test of Date
(After Flax/After Clustering/Outlier Removed)
R=0.70115
Estimated
1900
1850
1800
1750
1700
1650
1600
1450 1500 1550 1600 1650 1700 1750 1800
Specified

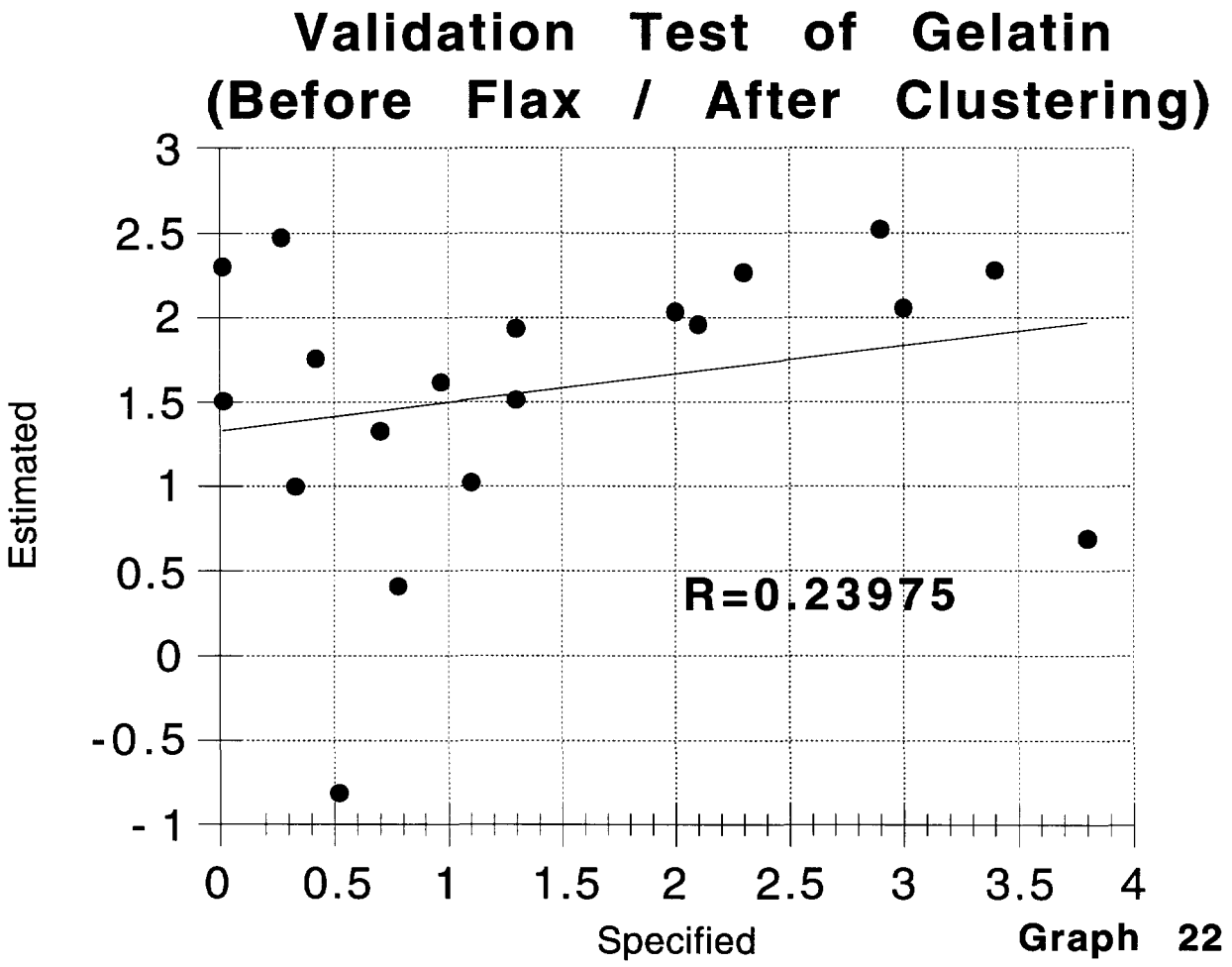
Graph 20B


Gelatin
(Before Flax / After Clustering)
2.5
2
1.5
1
0.5
0
Estimated
R=0.3614
0
1
2
3
4
5
Specified
Graph 21

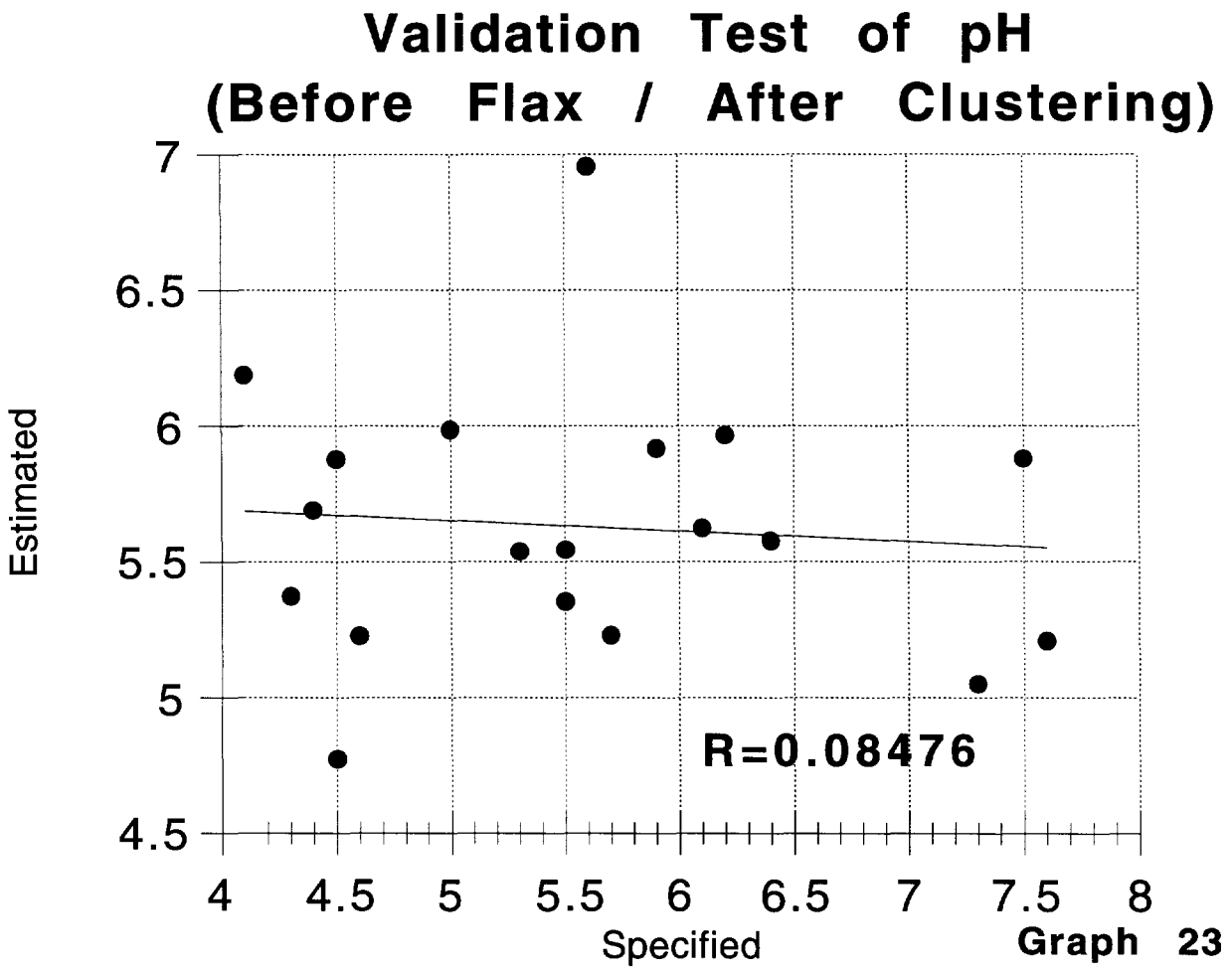
CORRELATIONS WITH NO FLAX ADDED TO CALIBRATION AND AFTER CLUSTERING

PROPERTY	PCR FACTOR	CORRELATION COEFFICIENT	MULTIPLE CORRELATION (IF APPLICABLE)
GELATIN	3	0.3614	
pH	2	-0.3960	
LIGHT	3	0.4374	
CALCIUM	1	-0.3123	
SULFUR	1 2 3 4	0.5890 -0.2416 -0.6785 -0.7481	0.9965
POTASSIUM	1 4 5	0.4938 -0.4457 -0.4910	0.8170
IRON	2	-0.9199	
FLUORESCENCE	2 3 4 5	-0.3658 0.2633 -0.3137 -0.6279	0.8558
DATE	1	0.4241	

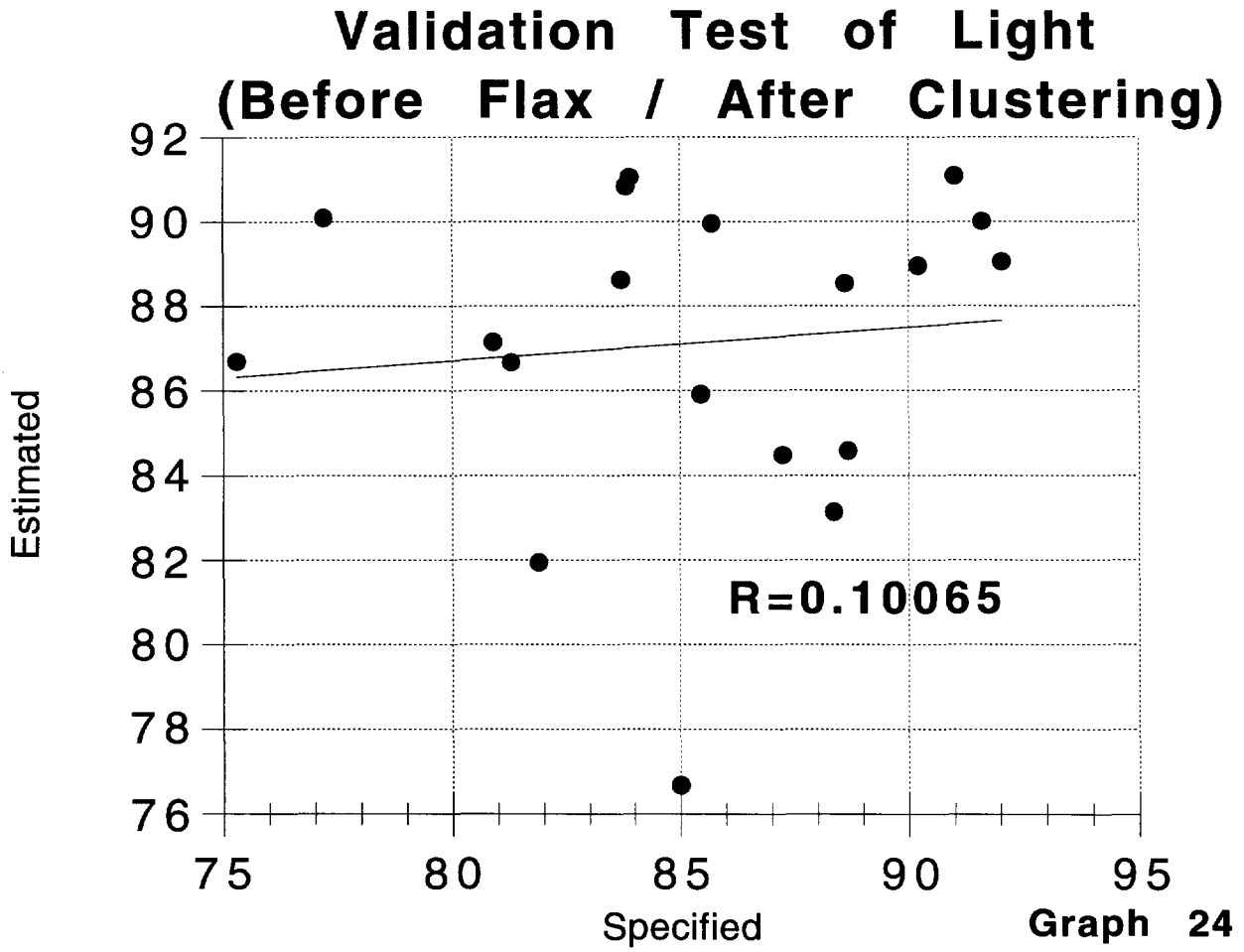
Table 4


Validation Test of Gelatin
(Before Flax / After Clustering)
Estimated
3
2.5
2
1.5
1
0.5
0
-0.5
- 1
0
0.5
1
1.5
2
2.5
3
3.5
4
Specified
R=0.23975

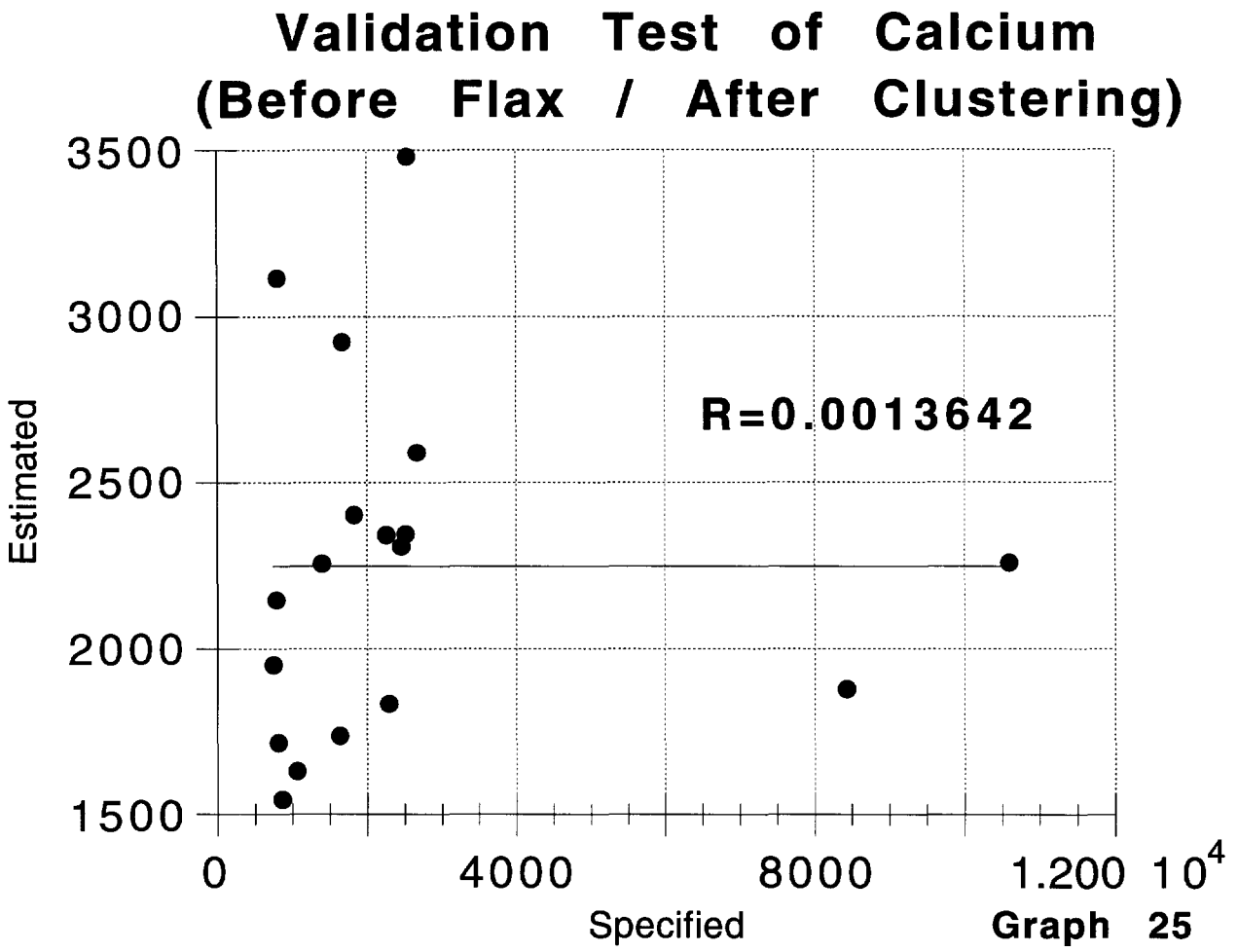
Graph 22


Validation Test of pH
(Before Flax / After Clustering)
Estimated
7
6.5
6
5.5
5
4.5
R=0.08476
4
4.5
5
5.5
6
6.5
7
7.5
8
Specified

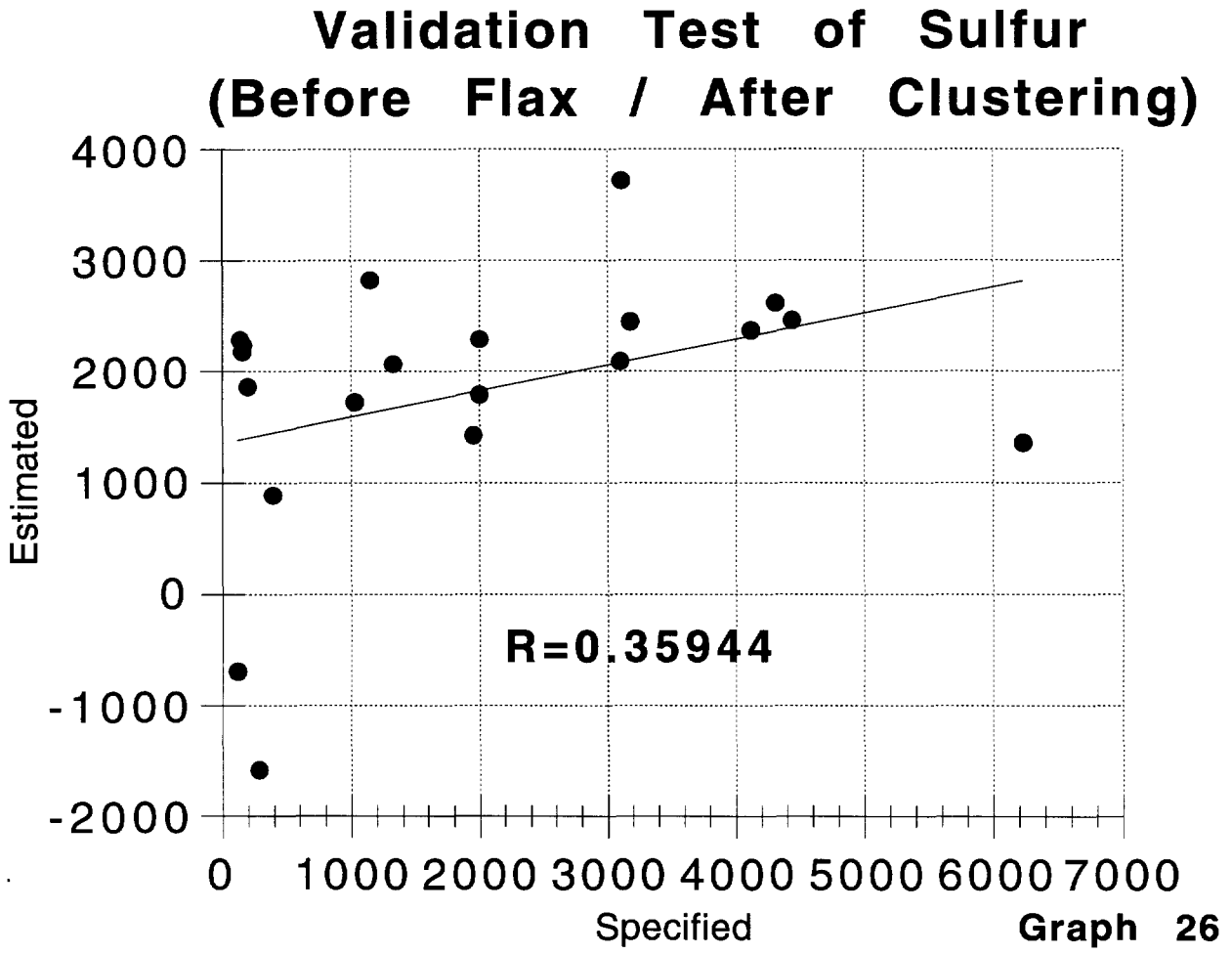
Graph 23


Validation Test of Light
(Before Flax / After Clustering)
92
90
88
86
84
82
80
78
76
Estimated
75
80
85
90
95
Specified
R=0.10065

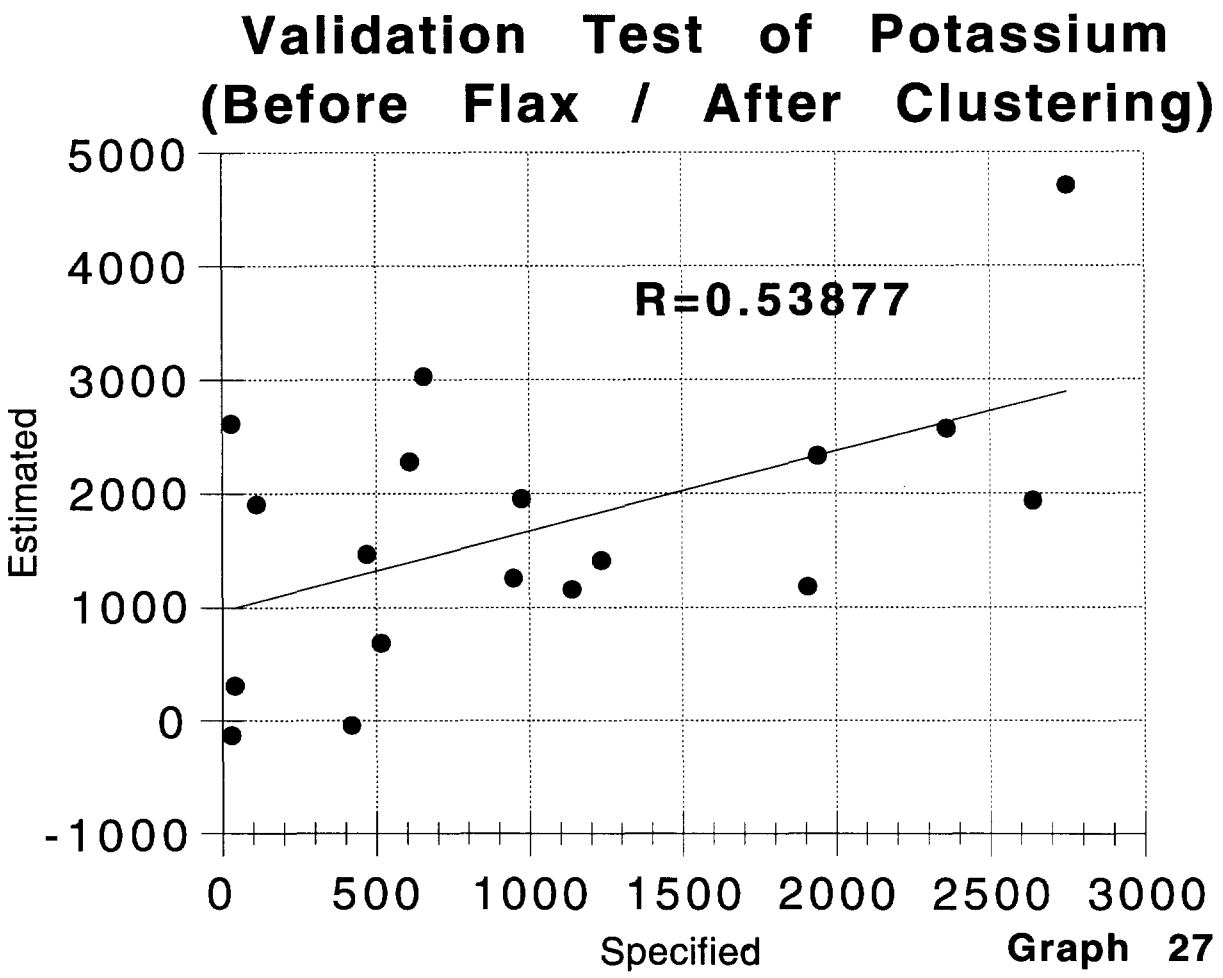
Graph 24


Validation Test of Calcium
(Before Flax / After Clustering)
3500
3000
2500
2000
1500
Estimated
R=0.0013642
0
4000
8000
1.200 10^4
Specified

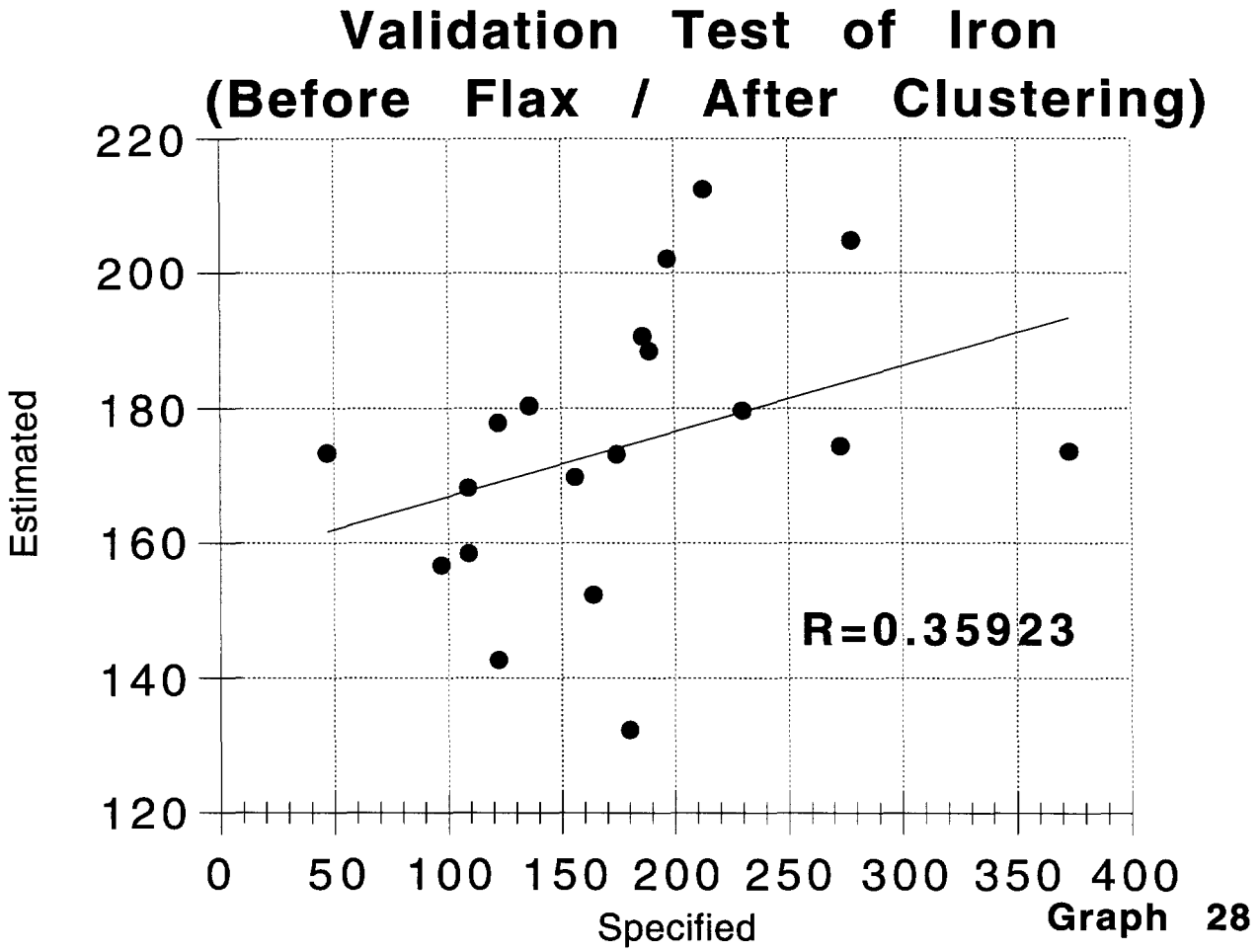
Graph 25


Validation Test of Sulfur
(Before Flax / After Clustering)
4000
3000
2000
1000
0
-1000
-2000
Estimated
R=0.35944
0
1000
2000
3000
4000
5000
6000
7000
Specified

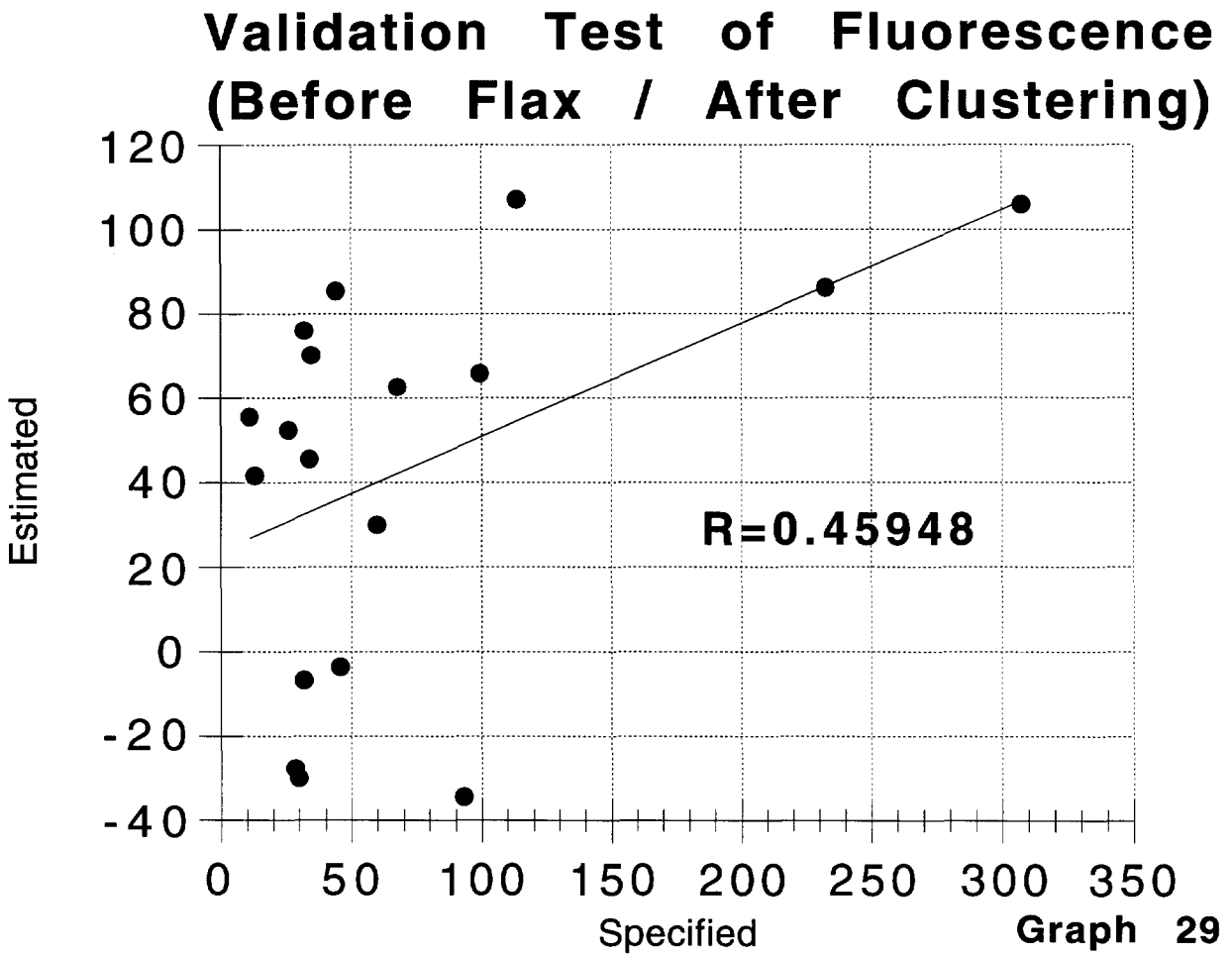
Graph 26


Validation Test of Potassium
(Before Flax / After Clustering)
R=0.53877
Estimated
Specified
5000
4000
3000
2000
1000
0
-1000
0
500
1000
1500
2000
2500
3000

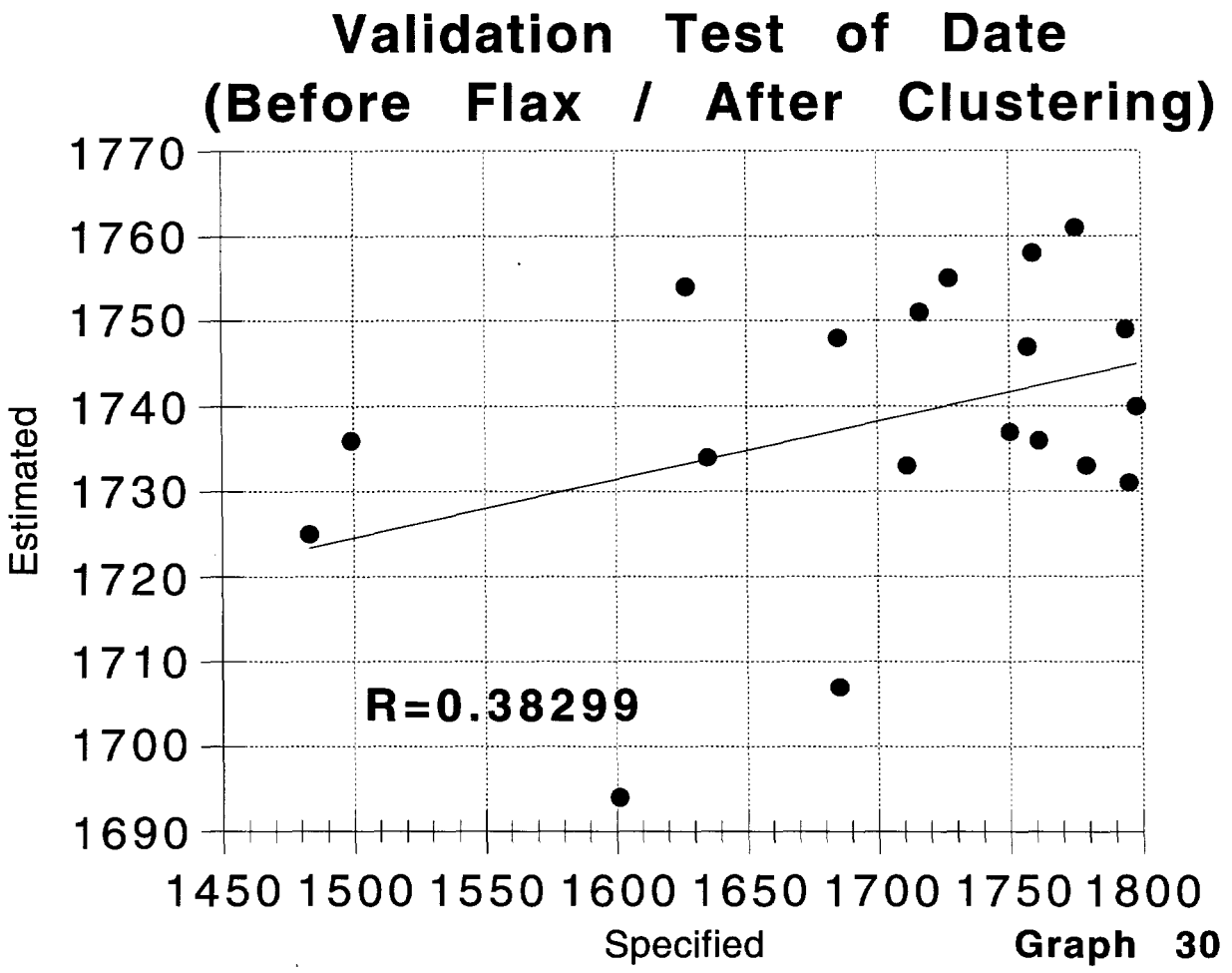
Graph 27


Validation Test of Iron
(Before Flax / After Clustering)
220
200
180
160
140
120
Estimated
0
50
100
150
200
250
300
350
400
Specified
R=0.35923

Graph 28


Validation Test of Fluorescence
(Before Flax / After Clustering)
Estimated
120
100
80
60
40
20
0
-20
-40
0
50
100
150
200
250
300
350
Specified
R=0.45948

Graph 29


Validation Test of Date
(Before Flax / After Clustering)
1770
1760
1750
1740
1730
1720
1710
1700
1690
Estimated
R=0.38299
1450 1500 1550 1600 1650 1700 1750 1800
Specified

Graph 30